Genetic Structure and Variability of H-FABP (HaeIII) Gene in Commercial Pig Breeds and Wild Boar in Slovakia

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Abstract
The heart fatty acid binding protein (*H-FABP*) has been decribed in several studies as a candidate gene for fat deposition (especially for IMF) in pigs. The genetic structure base on *H-FABP (Hae*III) polymorphism was analysed in a population of 51 animals (Mangalica, Large White, Landrace and Wild Boar). Genetic polymorphism of gene was detected by PCR-RFLP (*Hae*III). In the whole examined animal group the heterozygote genotype Dd was the most frequence (0.494)

Keywords: frequencies of alleles, genotype, H-FABP gene.

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1. Introduction

The *H-FABP* gene codes for a protein related to the intracellular transport of fatty acid and plays important role in the regulation of lipid metabolism. Given this physiological role, *H-FABP* has been considered to be an interesting candidate gene for intramuscular fat and backfat in pigs. Gerbens et al. [1] mapped *H-FABP* to pig chromosome 6 and detected three polymorphic sites in the porcine H-FABP gene, where the *Hinf*I site is located in the 5´upstream region, whereas *Hae*III and *Msp*I sites are about 300 bp apart in intron of the *H-FABP* gene. Authors found association between *H-FABP* gene and fat acreation, both IMF and BFT, in Duroc pigs. No significant association of *H-FABP* with IMF content could be detected in the new *Msp*I polymorphic site in Austrian animal group of Pietrain, Large White and Landrace [2]. Same results for Large White and Landrace were reported by [3] Urban et al. (2002). The effect of *H-FABP* – *Hinf*I polymorphism on the content of IMF, backfat thickness, the weight and percentage of major meat parts was no significant. Only in genotypes HH and hh the differences between LSM of phenotypic IMF values under study were close to the limit of significance [3]. Ovilo et al. [4] analyse the effect of the *H-FABP* polymorphism by Animal model where the *H-FABP* genotypes showed significant association on IMF, eye muscle area, but not on backfat thickness.

2. Materials and methods

Genotyping was performed in 51 randomly selected samples of Large White (n=15), Landrace (n= 14), Mangalica (n=10) and 12 Wild Boar (*Sus Scrofa Scrofa*). Large White and Landrace are representing the main breed kept on Slovakia, with typical combinated and ham confirmation. On the other hand, Mangalica are minor breed with typical fat utility with the minimum rate ot breeding. The PCR-RFLP methods by [1] were used to detect the genotypes of the *H-FABP* gene. Genomic DNA was extracted from the blood of 39 randomly selected commercial pigs (Mangalica 10, Large White 15, and Landrace 14) and 12 Wild Boar (*Sus Scrofa Scrofa*) by Wizard kit (Promega). Primers were designed from [1]

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published porcine *H-FABP* (*Hae*III) primers. The PCR reaction was performed in 25µl reaction using 100 ng porcine genomic DNA, 0, 2 µM each primer, 2,0 mM $MgCl_2$, 0,2 mM dNTP, 1,25 U of Go*Taq* polymerase (Promega) in standard PCR buffer. After an initial 95 °C (2 min.) denaturation step the PCR was carried out at 95 °C (30s.), 57 °C (30 s.), 72 °C (1,30 min.), for 30 cycles.

The 850 bp amplimer was cut with 1 units of *Hae*III restriction enzyme (Fermentas). PCR restriction fragments (allele D 850 bp and allele d 450 + 400 bp) were separated on 2% agarose gel.

3. Results and discussion

After the PCR reaction and restriction digestion with *Hae*III the restriction fragments were loaded on 2% agarose gel (Figure I).

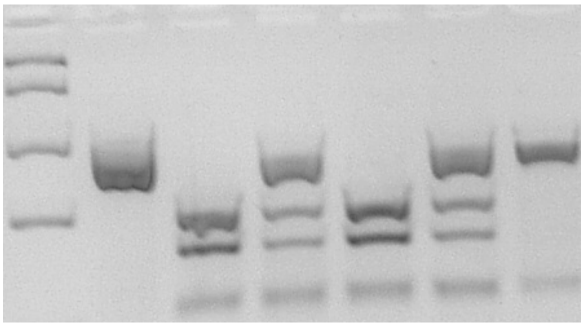

Figure 1. Representative results of PCR-RFLP polymorphism for *H-FABP* (*Hae*III) gene. Lane 1: marker (Fermentas Midlle Range), lane 2: PCR product (850 bp), lane 3, 5: dd (450 + 450 bp), lane 4, 6: Dd (850 + 450 +400 bp), lane 7: DD (850 bp).

Genetic structure of pig *H-FABP* gene is presented in Table 1. In the group of 51 randomly selected commercial purebred and Wild Boar population three genotypes were detected combination The heterozygote genotype Dd had the highest frequency (0.494), while the homozygotes genotype DD had the lowest frequency (0.194).

The same genotype structure (Dd = 0.5 and 0.49, DD 0.18 and 0.15 respectively) were detected in groups of Large White and Landrace. The similary results were reported by [1], when from 20 estimated Dutch Landrace, heterozygote genotype Dd was detected in 11 cases. The frequency of genotypes DD and Dd in the hybrid Hampshire x Duroc x Large White was identic, while genotype dd was detected only in a few cases [5].

In the group of Mangalica we detected only two genotype combination (DD, Dd). The homozygote genotype DD had the highest frequency (0.81) in Mangalica, while in other breeds (LW, L, WB) genotype DD was detected only in one case. On the other hand, in the Wild Boar population homozygote genotype dd was the most frequence (0.766), compare to other breeds. Heterozygote genotype Dd was detected only in one case, same as reported by [1] in the group of Wild Boar.

In the estimated population acordance with genotype structure, frequency of allele D was 0.441, opposite to results reported by [6]. Authors in a Berksihre, Duroc, Landrace and Hampshire population detected higher frequency of D allele (0.63). In the group of Wild Boar we detected low frequency of D alelle (0.125). The same resultes for Wild Boar were reported by [1], (D = 0.1). In the case of Landrace the frequency of allele D was higher (0.39), which coresponding with results reported by [1] for Dutch Landrace (D = 0.32).

According to results of genetic structure we estimated descriptive measure of genetic variability and allele effectivity (H_{obs}, H_{exp}, PIC, EA, F_S) for *H-FABP* gene, presented in Table 2. In the group examined as whole, the observed and expected heterozygosity were almost identic (0.451 and 0.493 respectively). These results corelated with the value of coefficient of inbreeding Fs (+ 0.087), indicated minimum increase in homozygotes phenotypes. In the case of Large White and Landrace breeds the expected heterozygosity were lower than observed, that coresponding with the decrease in homozygotes phenotypes indicated by values of Fs. On the other hand Wild Boar population showed the higher expected heterozygosity than observed, with the increase in homozygotes phenotypes (Fs = +0.63). Values of PIC (0.372) is lower than limit value compare to value of EA (0.198), that is near to limit value. The results showed a moderate reduction of *H-FABP* polymorphism in estimated population. Values of PIC and EA showed high polymorphism in groups of Large White and Landrace, opposite to considerable reduction of *H-FABP* polymorphism in groups of Mangalica and Wild Boar.

Table 1. Genetic structure of *H-FABP(Hae*III*)*

Breed	N	Frequencies of genotype			Frequencies of alleles		s
		DD	Dd	dd	D	d	
LW	15	0.18	0.5	0.32	0.43	0.57	0.00817
L	14	0.15	0.48	0.37	0.39	0.61	0.0085
M	10	0.81	0.09	0.01	0.9	0.1	0.0045
Wild Boar	12	0.016	0.218	0.766	0.125	0.875	0.00456
Total	59	0.194	0.494	0.312	0.441	0.559	0.0024

Table 2. Genetic variability of *H-FABP (Hae*III*)* gene

Breed	Heterozygosity		F_S	PIC	EA
	H_{exp}	H_{obs}			
LW	0.49	0.73	-0.46	0.370	2.0
L	0.048	0.64	-0.33	0.363	1.92
M	0.18	0.2	-0.11	0.164	1.22
Wild Boar	0.219	0.08	+0.63	0.194	1.28
Total	0.493	0.451	+0.087	0.372	1.98

4. Conclusions

Sample of 51 individual animals selected from commercial pig breeds has been evaluated by PCR-RFLP method for H-FAPB (HaeIII) gene polymorphism. Statistically significant differences in allele d frequencies have been found. Whole evaluated sample has high degree (0494) of the heterozygous genotype Dd presence. In the future its necessary to increase the number of animals to prove the association of these gene to production traits, especially to content of IMF and backfat thicknes.

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References

1. Gerbens, F., Rettenberg, G., Lenstra, J. A., Veerkamp, J. H., Pas, M. F. W., Characterization chromosomal localization, and genetic variation of the porcine heart fatty acid-binding protein gene. In: Mammalian Genome, 1997, 8, 328–332

2. Nechtelberger, D., Pires, V., Soolkner, J., Intramuscular fat content and genetic variants at H-FABP loci in Austrian pigs. In: J. Anim. Sci., 2001, 79, 2798–2804

3. Urban, T., Mikolášová, R., Kuciel, J., Ernst, M., Ingr, G., A study of associations of the H-FABP genotypes with fat and meat production of pigs. In: J. Appl. Genet., 2002, 43, 505–509

4. Óvilo, C., Oliver, M. A., Noguera, J. L., Clop, A., Barragan, C., Varona, L., Rodriguez, M. C., Toro, M., Sanchez, A., Perez-Enciso, M., Silio, L., Test for positional candidate genes for body composition on pig chromosome. In: Genet. Sel. Evol., 2002, 34, 465–479

5. Ye, X., Robinson, J. A. B., Jiang, Z., Verrinder Gibbins, A. M., Gibson J. P., Polymorphism of histone deacetylase 1 and 3 genes and fatty acid-binding protein 3 and 4 genes and thier associations with economic traits in swine. In: 7th World Congress on Genetics Applied to Livestock Production, August 19 – 23, 2002, pp. 123

6. Emnett, R., Moeller, S., Irwin, K., Rothschild, M. F., Plastow, G., Goodwin, R., Association Studies With Leptin Receptor, Melanocortin-4 Receptor, Melanocortin-5 Receptor, and Peroxisome Proliferator Activated Receptor-γ. In: Eastridge, M. L., Bacon, W. L., Knipe, C. L., Meeker, D. L., Turner, T. B., and Zartman, D. L. Research and Reviews: Swine, 2001, (OARDC Special Circular; 185), pp 57–63