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Characterization, chromosomal localization, and genetic variation of the porcine heart fatty acid-binding protein gene

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Abstract. The purpose of this study was to detect genetic variation in the porcine *H-FABP* gene, a candidate gene for meat quality traits in pigs. Lambda phages containing the porcine *H-FABP* gene were isolated by plaque hybridization with human H-FABP cDNA. The coding and flanking intronic sequences of the porcine *H-FABP* gene were determined as well as 1.6 kb of the 5′ upstream region. The various potential regulatory sequences in this region are in accordance with the function and expression of the protein in muscle and mammary tissue. Furthermore, comparison with the homolog region of the mouse identified a highly conserved 13-bp element (CTTCCT [A/C] TTTCGG) that may be involved in regulation of expression. The porcine *H-FABP* gene was localized on Chromosome (Chr) 6 by porcine sequence-specific PCR on DNA from a pig/rodent cell hybrid panel. In addition, part of the *H-FABP* gene was screened for genetic variation by PCR-RFLP analysis. Three PCR-RFLPs were detected, one in the upstream region (*Hin*fI) and two in the second intron (*Hae*III and *Msp*I). In most pig breeds the corresponding alleles have a variable distribution, possibly a consequence of selective breeding. This genetic variation will enable us to investigate the role of the *H-FABP* locus in porcine production and meat quality traits.

Introduction

One objective in pig breeding programs is the reduction of fat in the carcass to meet the consumers' demands for lean meat. Generally, fat reduction is surveyed as a decrease in backfat thickness. However, other fat depots such as the intramuscular fat (IMF) are reduced as well. Further reduction of this IMF would be undesirable because it is the main fat depot in meat and is positively correlated with meat quality traits (Wood et al. 1988). Hovenier et al. (1992) showed that IMF reduction is not completely correlated with backfat reduction, so both traits can be treated separately. Since IMF is hardly measurable in living animals, a (genetic) marker for this trait is necessary to exclude this depot from further reduction. Recently, it has been statistically shown that a major gene for IMF deposition is present in pigs (Janss et al. 1994), but the location and mode of action of this gene is still unknown. A candidate for this major gene may be the gene that encodes for heart fatty acid-binding protein (H-FABP).

Fatty acid-binding proteins (FABPs) are small intracellular proteins involved in fatty acid transport from the plasma membrane to the sites of β oxidation and/or triacylglycerol or phospholipid synthesis (Veerkamp and Maatman 1995). Furthermore, FABPs may modulate the intracellular fatty acid concentration (Veerkamp et al. 1993) and in this manner regulate various cellular processes and lipid metabolism in particular. FABPs are members of a family of intracellular lipid-binding proteins of at least eight structurally distinct types: adipocyte, brain, epidermal, heart, intestinal, ileal, liver, and myelin.

The heart type FABP (H-FABP) is a 15-kDa protein present in several tissues with a high demand for fatty acids such as cardiac and skeletal muscle and lactating mammary gland. Recently it became clear that mammary-derived growth inhibitor (MDGI), a protein that inhibits tumor cell growth (Bohmer et al. 1987), is in fact a mixture of H-FABP and adipocyte-type FABP (Specht et al. 1996). The murine *H-FABP* gene has been isolated and contains four exons encoding 24, 58, 34, and 17 amino acids, respectively (Treuner et al. 1994).

The aim of our studies is to look for a possible relationship between genetic heterogeneity in the *H-FABP* gene and variation in IMF deposition in pigs. To initiate this, we isolated and characterized the porcine *H-FABP* gene and determined its chromosomal localization. In particular, genetic variation of this gene was identified, which can be used for future association studies or linkage analysis with production and meat quality traits.

Materials and methods

Isolation of H-FABP containing phage clones. A porcine genomic DNA EMBL3/SP6/T7 lambda library (Clontech Laboratories Inc., Palo Alto, Calif.) was screened by plaque hybridization (Sambrook et al. 1989) to human H-FABP cDNA in the pSP65 vector (Peeters et al. 1991) labeled with [α-^{32}P]dCTP by nick translation (Sambrook et al. 1989). Briefly, 500,000 plaques were transferred to replica nitrocellulose filters and incubated in denaturation buffer (1.5 M NaCl/0.5 M NaOH) for 2 min, neutralization buffer (1.5 M NaCl/0.5 M Tris-HCl, pH 8.0) for 5 min, and fixation buffer [0.2 M Tris-HCl, pH 7.5/2 × SSC(0.3 M NaCl, 0.03 M sodium citrate)] for 30 s. The filters were air-dried and baked at 80°C for 2 h. The filters were prehybridized [6 × SSC/0.5% (wt/vol) SDS/5 × Denhardt's and 100 μg/ml NaOH-treated salmon sperm DNA] for 2 h at 67°C and hybridized at 67°C overnight in the same buffer containing the radioactive probe. The filters were washed four times with 2 × SSC, 0.1% (wt/vol) SDS for 30 min at room temperature.

Twenty plaques that showed positive signals on both replica filters were purified by two additional rounds of low-density plaque hybridization. Phage DNA was isolated by the plate lysate method (Sambrook et al. 1989).

Polymerase chain reactions. PCR amplifications were performed on 1 μl of a 1:1000 dilution of phage DNA preparations or 50 ng of genomic DNA in 50 μl containing 0.2 units Super Tth polymerase (SphaeroQ,

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The nucleotide sequence data reported in this paper have been submitted to the EMBL database and have been assigned the accession numbers X98555, X98556, X98557, and X98558.

Leiden, The Netherlands) in 10 mM Tris-HCl (pH 9.0)/50 mM KCl/1.5 mM $MgCl_2$/0.1% (wt/vol) gelatin/1% Triton X-100/0.5 μM of each primer (Pharmacia Biotechnologies, Uppsala, Sweden) and 0.2 mM of each dNTP (Boehringer Mannheim, Mannheim, Germany). After 3 min of denaturation at 94°C, 33 cycles of amplification were carried out: 94°C for 1 min, the indicated annealing temperature (Tables 3 and 4) for 1 min, and 72°C for the time considering the length of the expected fragment (ca 1 min for every kb).

DNA sequence analysis. PCR#1 (Table 3) was performed on DNA of the purified phage clones to identify the clones containing the *H-FABP* gene. Two positive phage clones were used to subclone the *H-FABP* gene. Therefore, the *Sac*I and *Kpn*I (Boehringer Mannheim) restriction digestion fragments of the phage DNA, containing the 5′ upstream region and exon 1 through exon 3, were subcloned in pBS. Intron 3 and exon 4 were amplified with porcine genomic DNA as template and primers from PCR#2 (Table 3). The 3′ untranslated region was amplified with porcine muscle cDNA as template and specific primers for porcine H-FABP exon 1 or 3 (Table 3) in combination with the anchor primer from the 5′/3′ RACE-PCR kit (Boehringer Mannheim). PCR products were cloned in the pT7Blue vector (Novagen Inc., Madison, Wis., USA). Products of two independent PCR reactions were cloned to identify errors by the Super Tth polymerase upon sequence analysis.

Recombinant plasmid DNA from H-FABP clones was purified with the Wizard Maxiprep kit (Promega). The nucleotide sequence was determined by dideoxy sequencing, either by cycle sequencing (Perkin Elmer, Foster City, Calif., USA) or autoread sequencing (Pharmacia Biotechnologies, Uppsala, Sweden) and the analysis was performed on a ABI 373 (Perkin Elmer, Foster City, CA, USA) or ALF DNA sequenator (Pharmacia Biotechnologies, Uppsala, Sweden), respectively. The DNA sequence was analyzed by the Genetics Computer Group (University of Wisconsin, Madison, Wis, USA) software packages.

Chromosomal localization. Two independently established pig/rodent somatic cell hybrid panels (Panel A, Rettenberger et al. 1996; Panel B, Zijlstra et al. 1994) were used to map the *H-FABP* gene to a specific chromosome by PCR. DNA (100 ng) from each cell hybrid containing porcine chromosomes in various combinations was used in the PCR#3 (Table 3) reaction, which unambiguously amplified porcine H-FABP intron 3 sequences.

Concordancy and correlation were statistically evaluated as described by Chevalet and Corpet (1986). The distribution patterns of the PCR signals for porcine H-FABP were compared with the distribution patterns of the pig chromosomes and of reference loci of individual pig chromosomes. A marker is syntenic with a chromosome or reference locus with a probability of 97.5% if the ϕ is >0.74 for 20 hybrid lines. Synteny can be excluded if ϕ is <0.59.

RFLP screening. Porcine genomic DNA was isolated as described (Sambrook et al. 1989) from EDTA-treated blood stored at −80°C. Genomic DNA (100 ng) was used for PCR amplification in 50 μl reaction mixture as described before. The primer sequences and its corresponding product size and annealing temperature for each combination are given in Table 4. Fifteen μl of the PCR reaction was used for restriction digestion with two units of *Hae*III, *Hin*fI, or *Msp*I (Boehringer Mannheim) in a total volume of 20 μl. *Msp*I digestion was carried out directly in the PCR buffer; *Hae*III and *Hin*fI digestions were carried out upon addition of the recommended concentrated reaction buffer. Restriction digestion fragments were loaded on a 2% (MspI) or 3% (HaeIII and HinfI) agarose (Sigma, St Louis, Mo., USA) gel. After electrophoresis the RFLP patterns were scored by two persons, independently. Allele frequencies between breeds were compared with a binomial model with a significance treshold of 95%. Genotype distributions within breeds were tested for Hardy-Weinberg equilibrium as described by Falconer and Mackay (1996).

Results

Porcine H-FABP gene sequence analysis. The H-FABP gene sequence was determined including 1.6 kb of the upstream regulatory region and 0.2 kb of the 3′ untranslated region (Fig. 1). To do this, three phage clones that contained the *H-FABP* gene were isolated from the porcine genomic DNA library by plaque hybridization. Subsequently, DNA from these phage clones was cloned and subjected to sequence analysis. The region downstream of exon 3 was isolated as PCR fragments amplified on porcine genomic DNA and muscle cDNA.

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ggcttccttctcagattccgaagagcgccttgaggccaggaaaggggactggtc
ctgccagccccggggagggattcggatcccgggtacccgtgctgccgggcgcct
acgggctgcccctttcctctttcggcaggctgggggatgggcgatcaacccggcg
aggcagcgcctgcatggggcttcctatttcggagcgggggcgtacgccacgcc
tcgtcacgtgacgctagggccatttaaagcggtagcgcgggctgggagccgccg
gtcctggaatttttgcgcgcctgttctgtcgtctctttctcagcctagcccagc
ctcaccATGGTGGACGCCTTCGCGGGCACCTGGAAGCTAGTGGACAGCAAGAAT
       M  V  D  A  F  A  G  T  W  K  L  V  D  S  K  N

TTCGATGACTACATGAAGTCAATTGgtgag<4.2 kb>ctcagGTGTGGGTTTT
 F  D  D  Y  M  K  S  I                       G  V  G  F

GCCACCAGGCAGGTGGCCAACATGACCAAGCCTACCACAATCATCGAAGTGAAT
 A  T  R  Q  V  A  N  M  T  K  P  T  T  I  I  E  V  N

GGGGACACAATCATCATAAAAACACAAAGCACCTTCAAGAGCACAGAGATCAGC
 G  D  T  I  I  I  K  T  Q  S  T  F  K  S  T  E  I  S

TTCAAGCTGGGAGTGGAGTTTGATGAGACAACAGCAGATGACAGGAAGGTCAAG
 F  K  L  G  V  E  F  D  E  T  T  A  D  D  R  K  V  K

gtgag<2.5 kb>cacagTCCATTGTGACACTGGATGGAGGCAAACTTGTCCAC
                  S  I  V  T  L  D  G  G  K  L  V  H

CTGCAGAAGTGGAATGGACAAGAGACAACGCTTGTTCGGGAACTAGTTGATGGG
 L  Q  K  W  N  G  Q  E  T  T  L  V  R  E  L  V  D  G

AAACTCATCCTGgtaag<1.5 kb>tccagACACTCACCCATGGCAGTGCAGTT
 K  L  I  L                    T  L  T  H  G  S  A  V

TGCACTCGCACTTACGAGAAAGAGGCATGAcctgcccatcccttcgactgttcc
 C  T  R  T  Y  E  K  E  A stop

tgccaattggctactcctggactcagcaccagattgcctcatttttttcctctgg
cattttgtaaaaatctactttggggatattctcctggggtcaggttgcaccagc
ctrcgttcagttccggttcttgttgtgtatgttwgttttttttttaattgcatcc
aaagggtgctctgaggtcaataaaatagccaaggc*cacc*
```

Fig. 1. The porcine H-FABP gene sequence including 330 bp of the 5′ upstream region and 200 bp of the 3′ untranslated region. Exons are represented by bold capital letters, and the deduced amino acid sequence is shown underneath and numbered. The putative TATA-box, the polyadenylation signal in the 3′ UTR, and the polymorphic *Hin*fI site (GATTC) are depicted bold and underlined. The 13-nucleotide element is depicted double underlined. The size of the nondepicted intron sequences is shown between arrowheads. The two poly-A tail starting positions are indicated by asterisks.

The exons were identified in the porcine H-FABP gene sequence (Fig. 1) according to homology with known H-FABP sequences of cattle and mouse. The percentage of identity of the coding part of the porcine *H-FABP* gene with the homologous genes of other species is very high (Table 1) both for the DNA sequence as well as for the predicted amino acid sequence. The resulting four exons encoded in respective order 24, 58, 34, and 17 amino acids, and the sizes of the introns were about 4.2, 2.5, and 1.5 kb, respectively.

Sequence analysis of the 5′ regulatory region revealed a potential TATA box 92 bp upstream the ATG start codon (Fig. 1) and potential consensus binding sites for various transcription factors such as MYOD and hormone receptors (Table 2). In the 3′ untranslated region the consensus poly-A signal sequence was identified (Fig. 1).

Screening of the porcine genomic DNA library also yielded H-FABP

Table 1. Interspecies identity of the H-FABP encoding DNA sequence and the predicted amino acid sequence.

Species	Percentage identity with porcine H-FABP	
	DNA	Protein
Cattle[a]	92	92
Human[b]	91	90
Mouse[c]	84	87
Rat[d]	85	86

[a] Billich et al. 1988.
[b] Peeters et al. 1991.
[c] Binas et al. 1992.
[d] Claffey et al. 1987.

Table 2. Position of transcription factor binding sites in the porcine *H-FABP* gene upstream region.

Factor	Consensus sequence	Position
Activator protein (AP-1)	STGACTMA	−875
Activator protein (AP-2)	CCSCRGGC	−408
Activator protein (AP-3)	TGTGWWW	−1545, −714
CCAAT-EBP (C/EBP)	TKNNGYAAK	−1608, −1504, −1072, −665, −628
E-box	CANNTG	−1593, −1328, −1111, −1308, −768, −110
Growth hormone (GH-cse2)	AATAAAT	−1441
Glucocorticoids (GRE)	TGTTCT	−1000
Krox-24	GCGSGGGCG	−134
Mammary activ. factor (MAF)	GRRGSAAGK	−1134
Stat-5 (MGF)	TTCNNNGAA	−1178

pseudogene-like sequences as identified by the absence of intronic sequences. Two of these pseudogenes were analyzed by sequencing the PCR#2 (Table 3) amplification products. Furthermore, a pseudogene product amplified on porcine genomic DNA was also analyzed. Various nucleotide substitutions as well as frameshifts were detected between the *H-FABP* gene coding region and pseudogene sequences. All three pseudogene sequences were distinct from one another, and one contained a 27-bp internal duplication (data not shown; sequences submitted to EMBL database with accession No.'s X98555, X98556, and X98557).

Genomic location of the porcine H-FABP gene. The presence of the porcine *H-FABP* gene in two independently established pig/rodent cell hybrid panels, A and B, was tested by the porcine-specific PCR#3 (Table 3). The obtained data were compared with the known cytogenetic data of both panels and data from already mapped loci for each chromosome, the so-called reference loci, for panel A. The *H-FABP* gene could be assigned to pig Chr 6 because a single significant correlation (ϕ) was detected for both panels of 0.89 and 0.83, respectively. All other chromosomes were asyntenic for both panels because the correlation did not exceed 0.54.

Sites of genetic variation in the porcine H-FABP gene. Digestion of PCR (Table 4) products with the restriction digestion enzymes *Hae*III, *Hin*fI and *Msp*I revealed three fragment length polymorphisms (RFLP). The *Hin*fI site is located in the 5′ upstream region, whereas the *Hae*III and *Msp*I sites are about 300 bp apart in intron 2 of the H-FABP gene.

The frequencies of the different RFLP genotypes for various pig breeds were determined in unrelated animals (Table 5). The three polymorphisms are present in all breeds tested, except for the *Hae*III and *Msp*I RFLPs in the Hampshire and Meishan breed. The allele frequencies estimated for pig breeds represented by more than nine animals revealed significant differences between them (Table 5). Furthermore, in the Duroc breed the *Hin*fI allele frequency distribution is at disequilibrium.

Discussion

Although the fatty acid binding and antigenic crossreactivity of porcine H-FABP have been studied extensively (Paulussen et al. 1988), its amino acid sequence has not been known until now. We show that 86% to 92% of the predicted amino acid sequence of porcine H-FABP is identical to the H-FABP sequences of other mammalian species (Table 1). The higher similarity of porcine and human H-FABP, compared with rat H-FABP, is reflected also in the antigenic crossreactivity patterns (Paulussen et al. 1990).

The porcine *H-FABP* gene resembles the murine *H-FABP* gene (Treuner et al. 1994) in overall structure, although the porcine introns are larger. Furthermore, a potential TATA box (TTTAAA) and poly-A signal (AATAAA) are present. Analysis of the porcine H-FABP cDNA sequences revealed two poly-A tail start sites four nucleotides apart (Fig. 1). In fact, an alternative poly-A start site with a spacing of four nucleotides was previously reported as one of the differences between bovine H-FABP and MDGI cDNA sequences (Spener et al. 1990), but also corresponds to the differences between the murine *H-FABP* gene and a pseudogene-like sequence (Treuner et al. 1994).

H-FABP pseudogene-like sequences have been localized to murine Chrs 8, 10, and 17 (Heuckeroth et al. 1987; Treuner et al. 1994) and human Chr 13 (Veerkamp and Maatman 1995), respectively. Several clones that contained pseudogenes were isolated from the porcine genomic DNA library. On the basis of restriction digestion patterns of the clones, we estimated that the porcine genome contains at least three different *H-FABP* pseudogenes (data not shown). Three porcine pseudogenes from two different pigs were analyzed by sequencing a PCR product containing a part of the *H-FABP* pseudogene. Alignment of these sequences with the *H-FABP* gene sequence showed that all three had frameshift mutations, and one had an internal 27-bp duplication (data not shown). Moreover, we detected various nucleotide substitutions in all three porcine pseudogene fragments, whereas the murine *H-FABP* pseudogene differed by only three nucleotides from the complete murine H-FABP coding sequence. In mice no expression of the *H-FABP* pseudogene was detected in an array of tissues (Treuner et al. 1994). In conclusion, it is unlikely that the porcine pseudogenes are expressed because of the additional high degeneracy of these sequences.

Potential transcription factor binding sites have been found in the 5′ upstream region of the murine *H-FABP* gene (Treuner et al. 1994). These binding sites can also be found in the porcine *H-FABP* gene upstream region (Table 2) and are consistent with the reported tissue-specific expression or function of *H-FABP*. For

Table 3. The conditions for porcine *H-FABP* gene specific PCR reactions

PCR	Site[b]	Primer sequence	T_{ann}[a]	Size (kb)
#1	exon 1	5′GCCAGCATCACTATGGTGGACGCTTTC		
	exon 2	5′CTTAAAGCTGATCTCTGTGTTC	57	4.4
#2	exon 3	5′GGAGGCAAACTTGTTCACCTGC		
	exon 4	5′TCTTTCTCGTAAGTGCGAGTGC	57	1.6
#3	exon 3	5′GGAGGCAAACTTGTTCACCTGC		
	intron 3	5′GTACTGGGAGCACTCTTCACTC	62	1.5

[a] Annealing temperature (°C).
[b] Exon primers are based on human and mouse H-FABP cDNA sequences and the intron 3 primer on porcine H-FABP DNA sequence

Table 4. The PCR conditions for the PCR-RFLP detection assays.

RFLP	Primer	T_{ann}[a]	Size (bp)
*Hae*III/*Msp*I	5′ATTGCTTCGGTGTGTTTGAG		
	5′TCAGGAATGGGAGTTATTGG	57	850
*Hin*fI	5′GGACCCAAGATGCCTACGCCG		
	5′CTGCATCTTTGACCAAGAGG	57	700

[a] Annealing temperature (°C).

Table 5. Allele frequency and number of heterozygotes for the porcine H-FABP RFLPs in various pig breeds.

		Allele frequency and number of heterozygotes per pig breed[a]						
RFLP	Allele[b]	DL	DU	GY	HS	ME	PI	WP
*Msp*I	A	.98[c]	.40[d]	81[e]	1.0	1.0[c]	.90	.70
	Aa	1	4	11	0	0	1	3
*Hae*III	D	.32[c]	.40[c]	.31[c]	1.0	1.0[d]	50	.10
	Dd	11	4	17	0	0	1	1
*Hin*fI	H	.70[c]	.70[c,d]	.97[d]	.33	.45[c]	70	.90
	Hh	10	2	2	2	6	1	1
n[f]		20	10	34	6	11	5	5

[a] DL, Dutch Landrace; DU, Duroc, GY, Great Yorkshire; HS, Hampshire; ME, Meishan; PI, Pietrain; WP, Wild Pig
[b] Alleles represented by letters: A, 750 + 100 bp; a, 850 bp; D, 850 bp; d, 450 + 400 bp, H, 197 + 59 bp; h, 256 bp (besides other fragments).
[c,d,e] Data within a row lacking the same superscript letter differ ($P < 0.05$).
[f] The number of unrelated animals tested per breed.

instance, the presence of potential E-boxes, which direct expression to skeletal muscle cells upon binding of the myogenic MyoD protein family, could direct the *H-FABP* expression. On the other hand, *H-FABP* is highly expressed in cardiac tissue, but this expression is not regulated by these myogenic factors that are not present in cardiac myocytes (Olson et al. 1995).

General metabolic regulation of the *H-FABP* gene could be explained by the presence of potential binding sites for activator proteins (AP) 1, 2, and 3, Krox 24, and the glucocorticoid-responsive element, which also renders responsiveness for the estrogen and progesterone receptors. The significance of the potential C/EBP (CAAT enhancer binding protein) binding sites is unclear, since this protein is thought to be primarily expressed in adipocytes (MacDougald and Lane 1995).

Interestingly, a 13-nucleotide (CTTCCT[A/C]TTTCGG)-long element found twice in 250 base pairs preceding the ATG start codon of the porcine *H-FABP* gene (Fig. 1) was also found once in the murine *H-FABP* gene upstream region. This element has no homology to any potential binding site in a transcription factor database as well as in the GENBANK/EMBL nucleotide databases and probably represents a new element involved in transcriptional regulation.

The presence of potential Stat 5 binding sites would explain the pregnancy- and lactation-dependent expression of the *H-FABP* gene in the mammary gland (Binas et al. 1992). Namely, Stat 5 is a member of the Jak/Stat signal transduction pathway and is activated by the pregnancy-dependent hormone prolactin in the mouse mammary gland (Liu et al. 1995). This Stat 5 binding site is also detected in the promoters of various milk protein genes (Watson et al. 1991) that are expressed in late pregnancy and during lactation.

Two independent cell hybrid panels (Rettenberger et al. 1996 and Zijlstra et al. 1994) have been used for mapping. The porcine *H-FABP* gene was localized on Chr 6. The corresponding human and mouse H-FABP genes are localized on Chr 1p32-p33 (Peeters et al. 1991; Troxler et al. 1993) and Chr 4 (distal to *Lck;* Bahary et al. 1991), respectively, in a region that is highly conserved in evolution (Paszek et al. 1995). Several other genes that are mapped in this conserved region in mice and human, like ENO-1 and PGD (Yerle et al. 1995), are also found on porcine Chr 6. Indeed, heterologous chromosome painting of human chromosomes to the pig genome indicated that porcine Chr 6q21-26 is homologous to parts of human Chr 1 (Rettenberger et al. 1995).

In our search for genetic variation in the *H-FABP* gene, RFLPs were detected in the upstream region and intron 2. The subsequent genotypes show a diverse distribution in the pig breeds tested except in the Hampshire and Meishan breeds for the intron 2 specific RFLPs (Table 5). Interestingly, also wild pigs show heterogeneity for each RFLP.

Although for some breeds the sample size is very small, the observed difference in the genotype frequency distribution between breeds may be the result of selective breeding. However, a small number of founder animals for a population either by changed selection criteria or import, as for the western Meishan population, also changes the allele frequency.

The *Hin*fI allele frequency distribution is at disequilibrium in the Duroc breed, which may suggest selection pressure in favor of the H allele. Interestingly, the Duroc breed has a different *Msp*I allele frequency distribution than the Great Yorkshire and the Dutch Landrace breeds. The Duroc breed has a higher percentage of IMF and a lower growth rate compared with the Great Yorkshire breed (Hovenier et al. 1992), which, upon selection for growth rate, may have changed the allelic distribution of the H-FABP RFLPs. Currently we are investigating possible relations of the *H-FABP* genotypes with meat quality traits like IMF and the more general traits as fat deposition and lean growth.

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Patent. A patent (No. 96200855.3) was filed for the porcine H-FABP gene sequence, the described PCR-RFLP tests to detect genetic variation within it, and the use of these tests in breeding programs.

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