Novel polymorphism in the porcine Apolipoprotein B gene detected by single-strand conformation polymorphism analysis.

Toshimi Matsumoto¹, Rho Yamada¹,², Yoshiki Shimatsu³, Tomiji Akita⁴, Akira Onishi⁴, Eiji Kobayashi¹,⁴, and Mitsuru Minezawa⁴

¹ The STAFF institute, 446-1 Kamiyokoba, Ippaizuka, Tsukuba-shi, Ibaraki 305-0854, Japan
² Tukuba Research Laboratory, Hitachi Chemical Co. Ltd., Japan
³ SLA Research Park, Inc., 6598 Toyoda, Suwa-shi, Nagano 392-0016, Japan
⁴ National Institute of Animal Industry, 2 Ikenodai, Kukisaki-machi, Inashiki-gun, Ibaraki 305-0901, Japan

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Abstract We analyzed the porcine gene coding for Apolipoprotein B (APOB) to examine variation in the PRE-1 sequence of this gene using DNA samples from 10 pig breeds and populations, and two families. The sequences of the primers were selected from unique sequences flanking the PRE-1 sequence in intron 27 of the porcine APOB gene. Four different patterns were detected by SSCP analysis. No polymorphism was detected in 6 breeds, while the two miniature pig breeds and the Japanese Wild pig have unique alleles at the locus. For haplotype analysis of the pig APOB gene, two other markers described previously were genotyped. Allelic distribution consistent with the results of previous reports was obtained. These results provide the allelic frequencies of polymorphisms at the APOB loci in Japanese pig populations for the first time. The APOB-PREI locus will be a useful marker for linkage analysis for specific phenotypic traits. Alignment of these polymorphism and haplotype analyses will be required.

Key words : APOB, PRE-1, SSCP analysis, Polymorphism

Apolipoproteins are polypeptide carriers of cholesterol, triglycerides, and phospholipids in the circulation. Apolipoprotein B (APOB) plays an important role in lipoprotein flow. APOB is required for the secretion of very-low-density lipoprotein (VLDL) and chylomicrons by liver and intestine, respectively, and is also a primary component of low-density lipopro-
tein (LDL) as a ligand for the LDL receptor; it is derived in the circulation by lipolysis of VLDL (Kane, 1983). It then mediates the clearance of LDL from the bloodstream in mammals. Porcine APOB is highly polymorphic and eight isoforms, termed $LPB^1 - LPB^8$, have been recognized through immunological studies (Rapacz and Hasler-Rapacz, 1984). It was also found that pigs with certain APOB immunotypes develop hypercholesterolemia associated with atherosclerosis at an early age. The $LPB^5$ allele is associated with elevated levels of plasma LDL, resulting in part from a delayed clearance of LDL (Rapacz et al., 1986). Thus, APOB genetic variation significantly influences LDL metabolism in pigs and may be related not only to diseases but also to body fat content.

The porcine repetitive element-1 (PRE-1) sequence, which is reported to be a short interspersed element (SINE) throughout the porcine genome (Singer et al., 1987), has been identified in intron 27 of the APOB gene (Maeda et al., 1988). In humans, differences in SINE sequences have been detected by PCR-SSCP analysis (Orita et al., 1990). We report here a novel polymorphism in the PRE-1 sequence of the porcine APOB gene using SSCP analysis and the distribution of its haplotypes at APOB loci in several pig breeds in Japan.

**Materials and Methods**

**Animals and DNA extraction**

DNA samples from the following 10 pig breeds and populations, constituting 71 unrelated animals, were used: Large White (7 animals), Landrace (12), Duroc (9), Jinhua (8), Berkshire (7), Clown-mini (4), Meishan (9), Goettingen-mini (8), Ohmini-hybrid (3), and Japanese wild pig (4). Two families, one a cross of a Landrace dam and Large White sire and the other of a Large White dam and sire, were used for confirmation of inheritance. Genomic DNA was extracted from peripheral blood or ear tissue and was used as a template for PCR amplification.

**PCR amplification**

The sequences of the primers used for PCR amplification, selected from unique sequences flanking the PRE-1 sequence in intron 27 of the porcine APOB gene, were: 5'-CGTGACCTTAGTGCAGAATAG-3' (forward primer, 3140 to 3160, indicating the position in Maeda et al., 1988) ; and 5'-CTTTGTTGTAATGCAGCAGATA-3' (reverse primer, 3629 to 3609). PCR amplification was performed in 25 μl of a reaction mixture containing 50 ng of genomic DNA as a template, 0.5 mM of each primer, 200 mM of each dNTP, 1.25 U of Taq polymerase (Boehringer Mannheim), and the reaction buffer recommended by the manufacturer. Samples were overlaid with light mineral oil (Sigma), incubated at 95 °C for 3 min, then processed through 30 cycles of amplification, each consisting of 30 sec at 95 °C (denaturation), 1 min at 58 °C (annealing), and 1 min at 72 °C (extension), followed by a final incubation at 72 °C for 5 min using a Thermal Cycler (DNA Thermal Cycler 480, Perkin Elmer).

**SSCP analysis**

The PCR products (about 490 bp) were denatured at 95 °C for 5 min to conform a 3-D structure by single-strand DNA, separated by electrophoresis on a 7.5% polyacrylamide gel without glycerol (16×16×0.1 cm) in Tris-borate-EDTA buffer, and then detected by Silver staining (Silver staining kit, Bio Rad). Electrophoresis was performed at 10 W/gel for
Fig. 1. SSCP analysis of the PRE-1 sequence at the intron between exons 27 and 28. Electrophoretic patterns show the four SSCP of APOB-PRE1 locus in 12 unrelated individuals. Lanes 1-2, Large White; lanes 3-5, Goettingen-mini; lanes 6-9, Japanese Wild pig; lanes 10-12, Ohmini-hybrid. Lanes 6, 8, 9, genotype b/b; lanes 1, 2, 3, 5, 7, b/d; lane 4, a/d; lane 11, c/c; lanes 10 and 12, b/c.

2.5 hr at 15°C. For haplotype analysis of the pig APOB gene, two other polymorphisms, APOB-HindIII and APOB-ins/del, were genotyped using previously reported methods (Kaiser et al., 1993).

Results and Discussion

The PRE-1 region in the intron between exon 27 and 28 of the pig APOB gene was amplified by PCR using specific primers. Amplification of the pig genomic DNAs resulted in single fragments of the predicted size based on the positions of the primers in the gene (Maeda et al., 1988). Four different patterns of amplified fragments were detected by SSCP analysis. Figure 1 shows these SSCP patterns in 4 pig breeds. The electrophoretic type that exhibited the fastest-migrating bands was putatively termed “a”, and the others were termed, in order of decreasing migration speed, “b”, “c”, and “d”. Offspring from a heterozygous boar were “b/d” hybrid type shown in lane 1 in Figure 1; a homozygous sow was b/b type segregated into two heterozygous or homozygous parental types (Figure 2). This kind of segregation indicates the presence of codominant Mendelian alleles. Distribution of the alleles at the APOB-PRE1 locus in the 10 pig breeds and populations examined is shown in Table 1. No polymorphism was detected in 6 breeds (Landrace, Duroc, Berkshire, Clown-mini, Jinhua, and Meishan); their genotypes were all homozygous b/b type. The other four breeds examined in this study exhibited variations. The APOB-PRE1 locus has four alleles owing to differences in nucleotide substitution in the various pig breeds. It is very interesting for
Table 1. Allele frequencies of APOB genotypes in several breeds.

<table>
<thead>
<tr>
<th>Breeds</th>
<th>No. of Animals</th>
<th>PREI</th>
<th>HindIII</th>
<th>ins/del</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>a</td>
<td>b</td>
<td>c</td>
</tr>
<tr>
<td>Large White</td>
<td>7</td>
<td>0</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Landrace</td>
<td>12</td>
<td>0</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>Duroc</td>
<td>9</td>
<td>0</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Berkshire</td>
<td>7</td>
<td>0</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Meishan</td>
<td>9</td>
<td>0</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Jinhua</td>
<td>8</td>
<td>0</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Clown-mini</td>
<td>4</td>
<td>0</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Goettingen-mini</td>
<td>8</td>
<td>1</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Ohmini–hybrid</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Japanese Wild</td>
<td>4</td>
<td>0</td>
<td>7</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^{a)} + : \) allele digested with HindIII, \(- : \) allele not digested.

\(^{b)} + : \) allele with a 283-bp insertion, \(- : \) allele without insertion.

Characterization of miniature pigs that Goettingen–mini and Ohmini–hybrid pigs have unique alleles at this locus. Variations in the **APOB**–**PREI** locus were precisely found in the two miniature pigs of distinct origin. However, the Clown miniature pig exhibited alleles that were identical with Chinese and European breeds. Although new APOB allootypes have so far been found only in miniature pigs (Rapacz et al., 1994), no relationship between allootypes and DNA variation was apparent in this study. The porcine APOB gene has been localized by in situ hybridization (Solinas et al., 1992) and by linkage analysis (Ellegren, 1993) to chromosome 3. Therefore, the **APOB**–**PREI** locus will be also a useful marker for linkage analysis of pigs and/or analysis of relationships between genotype and specific phenotypic traits.

To date, 6 RFLPs and 3 PCR-based polymorphisms have been identified in the porcine APOB gene and an association between these polymorphisms and immunogenetical allootypes has been found (Maeda et al., 1988, Kaiser et al., 1993 Purcell et al., 1993). Distributions of the alleles at the **APOB**–**HindIII** and **APOB**–**ins/del** loci are also shown in Table 1. These results provide the allelic frequencies of polymorphisms at the **APOB** locus in Japanese pig populations for the first time. The allelic distribution of the **APOB**–**PREI** locus is different from both that of the **APOB**–**HindIII** and **APOB**–**ins/del** loci. The **APOB**–**HindIII** allele, corresponding to **LPB**\(^{4}\) or **LPB**\(^{7}\) (Maeda et al., 1988, Kaiser et al., 1993), was observed in only 2 populations, Ohmini–hybrid and Japanese Wild pigs. The frequency of the allele presented here agrees with that of Rapacz et al. (1982), who showed that the **LPB**\(^{7}\) allele was infrequent in most of the domesticated pig breeds and groups, but was found with high frequency in less domesticated pigs. It is interesting to find this allele in two unique populations that have never been selected for economic traits. The **APOB**–**ins/del**\(^{+}\) allele, corresponding to **LPB**\(^{5}\), was found in only one
animal each in three breeds, Duroc, Berkshire, and Goettingen. Rohrer et al. (1994) reported that an allele at the APOB-HindIII locus was found in their reference families. It was shown in Japanese pig populations of several breeds that almost all pigs from Chinese and European breeds were homozygotes in the alleles APOB-HindIII− and APOB-ins/del−. In breeds having specific alleles, distribution of allelic variation might be estimated as a characteristic of the breed.

Haplotype analysis of the three APOB loci indicated that animals examined in this study could be grouped into seven haplotypes. The most common haplotype, APOB-PRE1b−HindIII−-ins/del−, was found in 8 pig breeds examined. Animals with the APOB-ins/del+ allele had one haplotype that was similar to that of most animals with the APOB-HindIII− allele, APOB-PRE1b−HindIII−-ins/del+ and APOB-PRE1b−HindIII+ -ins/del−, respectively. Animals with APOB-PRE1d also had one haplotype, APOB-PRE1d-HindIII−-ins/del−. Other haplotypes were observed in pig groups bearing variant alleles at the APOB-PRE1 locus.

Although we examined variations in the porcine APOB gene, our findings and information regarding relationships between genetic variations were relatively poor. The determination of relationships among these variations is important for detection and evaluation of the function of the APOB gene. Because 8 immunological variations (Rapacz and Hasler-Rapacz, 1984), 6 RFLPs (Maeda et al., 1988), 3 PCR-based polymorphisms (Kaiser et al., 1993), and 4 variations of mononucleotide repeat (Ellegren, 1993) have been identified in the pig APOB gene, alignment of these polymorphisms and haplotype analysis will be required.

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References


