Rapid Communication: Mapping of the Titin (*TTN*) Gene to Pig Chromosome 15[1]

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Source and Description of Primers. The initial primers for the PCR were designed based on human DNA sequence (accession no. X92412; Kolmerer et al., 1996). The position of the forward and reverse primers corresponded to exon 3 and exon 5, respectively. These primers are expected to amplify a fragment of 1.93 kb from human DNA. A PCR fragment of 2.1 kb was amplified from porcine genomic DNA. Partial sequences were compared with the human sequence, which showed 90% (1,038/1,159 bp) nucleotide identity. The pig sequences (AF124849-50) were then used to design additional primers that amplified a 1.8-kb fragment of pig *TTN* and were used both for physical mapping using the somatic cell hybrid panel (**SCHP**) and for PCR-RFLP analysis.

Primer Sequences. The primers based on human sequence were as follows: 5-TAA AAC ATC ACA GGC ATC AGA-3′ (forward) and 5-AGT CAG ATC CAA ATT CAT TCC-3′ (reverse). The primers designed from amplified pig sequence were as follows: 5′-GTC AAG AAA TCA GAA GCC ACC-3′ (forward) and 5′-AAA ACT TTG CCC TCG TCA AT-3′ (reverse).

Method of Detection. The PCR was performed in 10-μL reactions including .5 U *Taq* DNA polymerase (Promega, Madison, WI), 1 × PCR buffer, 1.5 m*M* $MgCl_2$, .1 m*M* of each dNTP, 4 pmol of each primer, and 12.5 ng of porcine genomic DNA. The temperature conditions included initial denaturation at 94°C for 3 min, followed by 35 cycles at 94°C for 45 s, 63°C for 45 s, 72°C for 2 min and 15 s, and a final extension at 72°C for 5 min in a Robocycler (Stratagene, La Jolla, CA). The PCR products were electrophoresed on 1.0% agarose gels and visualized using ethidium bromide staining.

Description of Polymorphism. The 1.8-kb PCR product was searched for RFLP using a set of restriction enzymes on samples from several different pig breeds. Digestion with *Nla*IV revealed a polymorphism that resulted in two major allelic fragments of sizes 1.5 kb and 1.3 kb for alleles 1 and 2, respectively (Figure 1).

Pattern of Inheritance. Segregation consistent with autosomal Mendelian inheritance was observed in the Swedish Large-White × Wild Boar family that is included in the PiGMaP material (Archibald et al., 1995).

Allele Frequencies. A total of 73 animals from seven breeds and crosses were genotyped. Allele 1 was observed in Large White, Berkshire, and Duroc pigs in low frequency. Only allele 2 was detected in the other breeds evaluated (Table 1).

Chromosomal Location. The physical mapping was performed using a pig/rodent somatic cell hybrid panel comprising 27 cell lines (Yerle et al., 1996) and resulted in mapping of *TTN* to pig chromosome (SSC) 15q23–q26. The linkage results were obtained using two-point linkage analysis with the CRI-MAP program (Green et al., 1990) and the genotypes of PiGMaP animals (Archibald et al., 1995). The genetic mapping confirmed that the chromosome location was SSC15 and showed that *TTN* is linked to the following markers (centimorgans and two-point LOD score in parentheses): *S0148* (10.0, 6.02), *EAG* (10.0, 3.20), *DPP4* (0, 6.02), *S0088* (0, 5.42), and *S0284* (0, 5.42).

Comments. Titin is the third most abundant protein in muscle, after myosin and actin. It makes up approximately 10% of the combined muscle protein con-

Table 1. Frequency of *Titin* genotypes in seven different breeds

		Genotypes			Allele[a]	
Breed	n	1/1	1/2	2/2	1	2
Large White	24	.04	.25	.71	.17	.83
Hampshire	13	0	0	1.0	0	1.0
Berkshire	4	0	.25	.75	.12	.88
Meishan	11	0	0	1.0	0	1.0
Wild Boar	2	0	0	1.0	0	1.0
Landrace	8	0	0	1.0	0	1.0
Duroc	11	0	.36	.64	.18	.82
Total	73	.01	.15	.84	.09	.91

[a]1 = 1.5-kb *Nla*IV fragment; 2 = 1.3-kb *Nla*IV fragment.

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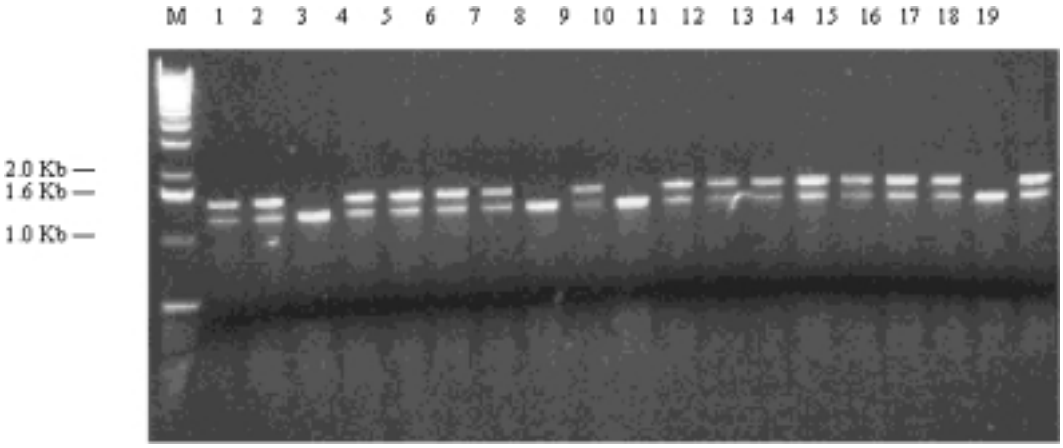

Figure 1. *TTN*-PCR-RFLP (*Nla*IV) segregation among F_2 offspring whose F_1 parents have genotypes 12 and 22. In lanes 1 to 19, upper and lower bands represent alleles 1 and 2, respectively.

tent and is called the giant protein of vertebrate striated muscle (Labeit et al., 1997). The portion of the gene examined in this study, including large parts of the intronic sequence, is highly conserved among species (Kolmerer et al., 1996).

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