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Identification and characterization of a new allele for the beta subunit of follicle-stimulating hormone in Chinese pig breeds*

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Summary

During evaluation of follicle-stimulating hormone-beta (*FSHB*) expression in anterior pituitary glands by an RNase protection assay (RPA), the expected fragment of 205 nucleotides at positions 759–963 was not detected in one boar that had moderate plasma and pituitary FSH concentrations. After subcloning and sequencing, mRNA from this boar lacked an 11-bp fragment (5′-CATTTGGAAAC-3′) at nucleotide positions 807–817 of the 3′-untranslated region (3′-UTR, *D* allele). Wild-type *FSHB* (*WT* allele) was present in pituitary RNA and genomic DNA in both Meishan (MS) and White Composite (WC) pigs; whereas the *D* allele was present only in MS pigs ($P < 0{\cdot}01$; 5/6 MS vs. 0/6 WC). Also, we found the *D* allele in five other Chinese breeds but absent in ten American Landrace, 11 Yorkshire and 17 Berkshire pigs. Additionally, the *D* allele had one silent nucleotide change in the coding region plus six, single nucleotide changes in the 3′-UTR.

Keywords: follicle-stimulating hormone beta, breed variation, swine

Follicle-stimulating hormone (FSH) belongs to a family of α/β heterodimeric glycoprotein hormones that includes luteinizing hormone, thyroid-stimulating hormone and chorionic gonadotropin. The common α-subunit and the β-subunit for FSH are each encoded by a separate gene. Expression of *FSHB* in anterior pituitary glands of boars correlates positively with blood and pituitary FSH concentrations (Zanella *et al.* 1996; Li *et al.* 1997; Li *et al.* 1998a). The current investigation was undertaken to understand the following. Why did a Meishan (MS) × White composite (WC) boar (no. 8) with moderate plasma FSH concentrations and *FSHB* mRNA, determined by RIA and RT-PCR, have no fragment corresponding to the expected size of *FSHB* by an RPA?

Three Chinese pig breeds (MS, Fengjing and Minzhu), a WC line (1/4 Chester White, 1/4 Landrace, 1/4 Large White, and 1/4 Yorkshire) and MS × WC crossbred pigs were randomly selected from populations at US Meat Animal Research Center (Table 1). Additional liver or skin samples were collected from American Landrace, Yorkshire and Berkshire pigs (Table 1). DNA samples of Erhualian, Xiang and Hainan pigs (Table 1) were provided from different farms in the People's Republic of China. Pigs were selected to maximize ancestral diversity.

FSH concentrations were determined in plasma and extracts of anterior pituitary glands (Zanella *et al.* 1996). Total RNA was isolated from pituitary glands by guanidine isothiocyanate extraction and CsCl centrifugation (Chirgwin *et al.* 1979). RNase protection assay was conducted as described previously (Li *et al.* 1997). Sense and antisense porcine *FSHB* and β-actin riboprobes were transcribed in the presence of [α-^{32}P] UTP with DNA-dependent T7 or SP6 RNA polymerases from the linearized plasmids with *Eco*RV or *Hind*III (Li *et al.* 1997).

In boar no. 8, no fragment corresponding to the expected size was detected by RPA, but a smaller fragment was observed. The RPA with antisense β-actin riboprobe indicated that RNA had not undergone degradation. This boar had moderate plasma FSH concentrations (236 ng/ml). Reverse-transcription and amplification of pituitary RNA from boar no. 8 with primers (sense: 5′-ATTTCCATCCCAAACCCC-3′; antisense: 5′-TCACCCATTCTTAGCCCATTTC-3′; Li *et al.* 1997) that spanned the protected region of the *FSHB* revealed an expected band (i.e. 205 bp) on ethidium bromide stained agarose gel for all males including boar no. 8. Subsequent sequence analysis of three independent clones indicated that an 11-bp fragment (5′-CATTTGGAAAC-3′; nucleotide positions 807–817) was deleted from *FSHB* mRNA of boar no.

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*The mention of names is necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the same by USDA implies no approval of the product to the exclusion of others that may also be suitable. GenBank accession number: AF134151.

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Table 1. Distribution of *FSHB* alleles in different breeds

Breed	N	*WT*/*WT**	*WT*/*D*†	*D*/*D*	F(*D*) allele
Meishan	6	1	3	2	0.58
Fengjing	5	2	2	1	0.40
Minzhu	8	3	4	1	0.38
Erhualian	6	2	2	2	0.50
Xiang	6	1	3	2	0.58
Hainan	6	4	2	0	0.17
WC	6	6	0	0	0
Amer. Landrace	10	10	0	0	0
Yorkshire	11	11	0	0	0
Berkshire	17	17	0	0	0

**WT*, wild type allele.
†*D*, deleted allele.

8 (*D* allele) compared to the reported allele (*WT* allele; Hirai *et al.* 1990).

When the amplified RT-PCR products were resolved on denaturing polyacrylamide gels, the frequency of *WT*/*WT*, *WT*/*D*, and *D*/*D* was 37·5%, 58·3% and 4·2%, respectively (Fig. 1A). An identical distribution was also obtained from genomic DNA, indicating that the 11-bp deletion was not post-transcriptional modification (Fig. 1B).

Genomic DNA isolated from liver or skin was amplified (Rohrer *et al.* 1994a) with *FSHB* sense (5′-ATTTCCATCCCAAACCCC-3′) and anti-sense (5′-TCACCCATTCTTAGCCCATTTC-3′) primers (Li *et al.* 1997). Both *WT* and *D* alleles were detected in Chinese breeds, but only the *WT* allele was detected in WC, American Landrace, Yorkshire and Berkshire pigs (Table 1).

Linkage analysis was conducted on 104 pigs in the USDA-MARC reference population (WC, Duroc, MS, Fengjing and Minzhu crosses, Rohrer *et al.* 1994b). This analysis employed the described marker to determine *D* and *WT* alleles, a microsatellite (Tn) located 4 kb 5′ to the TATA (Ellegren 1993) and a PCR-RFLP located within the first intron of *FSHB* (Rohrer *et al.* 1994a). No recombinants were detected between these three markers.

In males heterozygous for the *FSHB* alleles, expression levels of these two were estimated by RPA specific for *FSHB* and normalized by β-actin mRNA. The *D* allele was expressed at similar ($P > 0{\cdot}05$) intensity as the Wt allele within three independent groups of males. Within each group, expression of the total of the two alleles was positively correlated with pituitary and plasma FSH concentrations ($P < 0{\cdot}01$).

Sequencing of full-length cDNA's of the *D* allele from two unrelated boars, including boar no. 8, revealed that both animals had six additional nucleotide differences in the 3′-UTR compared to the *WT* allele (Hirai *et al.* 1990). In the coding region, a transitional mutation from ATC to ATT was detected for isoleucine at codon 16 (GenBank accession no. AF134151).

In summary, we identified a *FSHB* allele in Chinese breeds of pigs that had a 11-bp deletion in the 3′-UTR plus a silent change in the coding region. The *WT* allele was present in all breeds examined, but *D* allele was detected only in Chinese breeds. Furthermore, we demonstrated that *D* allele was expressed at a level similar to

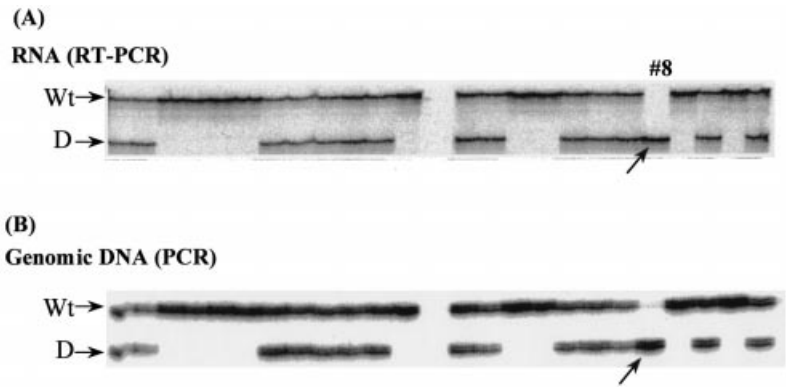

Fig. 1. Distribution of the *D* allele for *FSHB* in 24 MS × WC crossbred males. cDNA reverse-transcribed from RNA (A) or DNA (B) was amplified with *FSHB* specific primers. Among 24 MS × WC crossbred males screened by RT-PCR and PCR, only boar no. 8 was homozygous for the *D* allele.

WT allele in anterior pituitary glands of heterozygous males.

Presence of *D* allele at a high frequency in Chinese breeds and its absence in US breeds, especially Yorkshire and Berkshire, are puzzling. These two breeds, during their early development in late seventeenth and early eighteenth centuries, included crosses with Chinese pigs from the south region of China where Xiang and Hainan pigs were formed (Zhang *et al.* 1983; Jones 1998). Today the D allele exists in Xiang and Hainan pigs; therefore, we predict it was present in ancestors of these breeds. If so, why was this allele not transmitted into the European breeds when crossed with Chinese pigs? To date, no phenotypic traits are associated with the *D* allele. Li *et al.* (1998b) reported an association between an *FSHB* linked RFLP and litter size in Chinese pigs, but these findings conflict with our larger study of MS × WC gilts, in which no QTL for the components of litter size was identified on chromosome 2 where the *FSHB* gene resides (Rohrer *et al.* 1999).

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