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Single nucleotide polymorphism scanning and expression of the *FRZB* gene in pig populations

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ABSTRACT

Secreted frizzled-related protein 3 (sFRP3), encoded by the gene *FRZB*, is a member of the sFRP family with important roles in inhibition of the Wnt signalling pathway through competitive binding of the Wnt receptor. Here, we investigated pig *FRZB* as a candidate gene for growth traits and identified three polymorphic sites, an insertion (A-532B) and two SNPs (G636A and C650T) in its 5′-UTR. The genotype distributions of G636A and C650T were significantly different among mini-type indigenous (Diannan Small-ear and Tibetan), normal indigenous (Laiwu and Huai), and introduced (Large Yorkshire and Landrace) breeds. In semi-quantitative PCR expression analysis, expression of *FRZB* mRNA was abundant in tissues of hypophysis, *longissimus dorsi* muscle, and adipose tissues, and low in the heart, hypothalamus, and brain. Quantitative determination of mRNA level and protein expression analysis were corresponding. The results demonstrated that *FRZB* gene expression in *longissimus dorsi* muscle and liver tissue was significantly higher in Diannan Small-ear and Tibetan pigs than in the Large Yorkshire breed ($P < 0.05$); however, in back fat tissue, the expression was significantly higher in Diannan Small-ear pig than in Tibetan or Large Yorkshire breeds ($P < 0.05$). Given the known growth and fat characteristics of the breeds, these results indicate that *FRZB* expression has a negative association with muscle growth and a positive association with fat deposition. In conclusion, *FRZB* may be a major candidate gene for growth traits in pigs.

1. Introduction

Wnt proteins represent a large family of signalling molecules with significant roles in the regulation of cell proliferation, embryonic axis specification, and morphogenetic movements (Angers and Moon, 2009; Logan and Nusse, 2004). Secreted frizzled-related proteins (sFRPs) are structurally similar to the extracellular cysteine-rich domain (CRD) of the Wnt receptor, frizzled (Kong et al., 2012). For this reason, sFRPs can act as competitive inhibitors of Wnt signalling in the context of development and disease processes (Bovolenta et al., 2008). Wnt-dependent morphological alterations, such as axial duplication, are inhibited by sFRPs, suggesting that these molecules behave as dominant-negative receptors regulating the Wnt signalling pathway (Finch et al., 1997). sFRPs have been hypothesised as tumour suppressors (Jones and Jomary, 2002; Uren et al., 2000) and down-regulation of expression of the genes encoding them is associated with a number of diseases (Lee et al., 2004), including breast tumours (Wong et al., 2002; Zhou et al., 1998), and cervical and colorectal cancers (Ko et al., 2002; Suzuki et al., 2002).

The pig *FRZB* gene is located on chromosome 15 and consists of six exons and its function has not previously been investigated in pig populations. Chinese indigenous pig breeds, such as Diannan Small-ear pig (DSP), Tibetan pig (TP), Laiwu pig (LP) and Huai pig (HP), have lower growth rate, more fat deposition and better meat quality than introduced pig breeds (e.g. Yorkshire and Landrace) (Cheng, 1984; Plastow et al., 2005). Specially, the TP and the DSP are mini-type breeds that have distinct growth traits from the introduced breeds. In our previous RNA-seq experiment, we found that *FRZB* was differentially expressed in the *longissimus dorsi* muscle tissue of Diannan Small-ear pig and Large Yorkshire (Li et al., unpublished). Therefore, we hypothesised that pig *FRZB*, as a candidate gene for growth traits, may be differentially expressed in vivo and contain SNPs specific to different type pigs. The aims of this study were to discover SNPs associated with expression of the *FRZB* gene and contributing to growth traits and fat deposition in pigs, and to provide molecular information facilitating further research

Abbreviations: SNP, single nucleotide polymorphism scanning; sFRP3, secreted frizzled-related protein 3; *FRZB*, frizzled motif associated with bone development; UTR, untranslated region; CRD, extracellular cysteine-rich domain; DSP, Diannan Small-ear pig; TP, Tibetan pig; LW, Laiwu pig; HP, Huai pig; LY, Large Yorkshire; LL, Landrace; LD, *longissimus dorsi* muscle; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; PCR, Polymerase Chain Reaction; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; PBST, Phosphate Buffered Saline with Tween-20; *HPRT*, hypoxanthine phospho-ribosyl transferase; mRNA, messenger RNA; HWE, Hardy–Weinberg Equilibrium.

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Table 1
Target region, sequence, and amplicon size of the primers used for SNP identification in the pig *FRZB* gene.

Primer set	Target region	Forward primer sequence (5′ to 3′)	Reserve primer sequence (5′ to 3′)	Amplicon size (bp)
5′-FR1	−673/−224	TTGAAAGGGCAGGGTAGGA	AATCGGGTCACCAGAAGCA	450
5′-FR2	−476/572	CAGTGTGGTGTAATTTTCT	AGAGCAGGAGAAGAAGGGTGCAGTG	1048
5′-FR3	392/992	CCGAGGTGGGAAAGTGAA	CCCTGGAAGTGGACACAATG	601
FRZB-P1	Exon 1	CCCCAGAAGGCTTAGACG	AAGGGCGAGTAACAAGGACC	794
FRZB-P2	Exons 2–6	GGTTTATGACCGTGGCGTAT	CTCTGGGTGGAATCACTCTG	513
3′-FR1	3′-UTR	ACAGAGCATCAGTCTCGTTG	TCATCTTGTGTGTTGTTTCC	857
3′-FR2	3′-UTR	TTTTGGGCATTCTCCTCAC	CCAAATGACAAAGTGCAGGC	668

into the mechanism underlying the role of the *FRZB* gene in pig growth and meat quality traits.

2. Materials and methods

2.1. Experimental materials

Ear tissue samples from Diannan Small-ear pig (DSP, n = 59), Tibetan pig (TP, n = 77), Laiwu pig (LW, n = 51), Huai pig (HP, n = 26), Large Yorkshire (LY, n = 91), and Landrace (LL, n = 48) were collected for DNA extraction. DSP and TP are mini-type indigenous breeds native to Xishuang Banna, Yunnan (China), and Linzhi, Tibet, respectively. LW and HP are normal indigenous Chinese breeds native to Laiwu, Shandong, and Dingyuan, Anhui, respectively. LY and LL are introduced breeds that have characteristics of fast-growth and high-leanness. For extraction of total RNA and protein 24 castrated boars from the DSP, TP, and LY groups (8 each group) were slaughtered at 6 months of age. Tissue samples were collected from the liver, the *longissimus dorsi* muscle (LD), and back fat at the last rib, immediately frozen in liquid nitrogen, and then stored at −80 °C.

2.2. DNA, RNA, and protein extraction and cDNA preparation

Genomic DNA was isolated from ear tissue using standard procedures (Sambrook et al., 1989), dissolved in TE solution, and stored at −20 °C. Total RNA was isolated using TRIZOL® Reagent (Invitrogen, San Diego, CA, USA), checked for concentration and purity using a NanoDrop 2000 Biophotometer (Thermo scientific, USA; 260/280 nm absorbance ratio of 1.8–2.0 indicating a pure RNA sample), and separated by electrophoresis in a 1% agarose gel to verify integrity. After treatment with DNase I, a total amount of 2 μg RNA samples in a 20 μL reaction volume was reverse transcribed to cDNA using a SuperRT cDNA Kit (CWBIO Co Ltd., Beijing, China). Total protein was isolated from the LD, back fat, and liver using SDS Lysis Buffer (P0013B, Beyotime Ltd., China). Protein content was measured with the enhanced BCA protein assay kit (P0010, Beyotime, Ltd., China).

2.3. SNP screening and genotyping

Primers of the *FRZB* gene (KF112870) for SNP identification were designed using Primer Premier 5.0 software, amplifying coding regions (exons 1 to 6) and 5′ and 3′ flanking sequences of the gene. The targeted regions, primer sequences, and amplicon sizes are shown in Table 1. PCR products amplified from ten pigs in each group were pooled and sequenced directly to identify SNPs. Chromas Pro and DNAMAN6.0 were used to analyse the sequencing results. Genotypes of SNPs found by pooling sequencing were determined with individual PCR and sequencing.

2.4. Semi-quantitative RT-PCR

Heart, liver, lung, kidney, adipose, LD, hypothalamus, hypophysis, and brain tissues were collected from a TP. Primers of the *FRZB* gene (KF040089) were designed using Primer Premier 5.0 software to amplify products spanning an intron to avoid genomic DNA contamination. The primers were: 5′-GGAAGGTTCTATTGCTGAG-3′ and 5′-TTGCGTGC TTGCCGAGGGTTA-3′. We selected the housekeeping gene *β-actin* (DQ845171) as the internal standard (primers; 5′-TCTGGCACCACACC TTCTA-3′ and 5′-AAGGTCTCGAACATGATCTG-3′). Semi-quantitative RT-PCR was carried out using a thermal cycle (Eppendorf, German) in a 20.0 μL volume containing 0.3–1.5 μL cDNA, 10.0 μL 2× Power Taq PCR Master Mix (Bioteke, Beijing, China), 0.5 μL each of forward and reverse primers (10.0 nmol/μL), and added ddH_2O to the final volume. The PCR cycling protocol was an initial denaturation at 95 °C for 5 min, 22–28 cycles of denaturation at 95 °C for 20 s, annealing at 58–60 °C for 20 s, and extension at 72 °C for 20 s. The cycle number was optimised for all primer pairs (including those for *β-actin*) by analysing the intensity of the products amplified by 22 to 28 PCR cycles on agarose gels.

2.5. Quantitative analysis of FRZB mRNA expression

Real-time PCR was conducted on the Bio-Rad CFX96 System (Bio-Rad, USA), each reaction consisting of 10.0 μL 2× SYBR Green qPCR SuperMix (Transgen, Beijing, China), 1.0 μL cDNA, 0.4 μL each primer (10.0 nmol/μL) and supplemented with ddH_2O to a total volume of 20.0 μL. The real-time PCR programme started with a 20 second denaturation step at 95 °C, followed by 40 cycles of 5 s of denaturation at 95 °C and 15 s of annealing/elongation at 60 °C, during which fluorescence was measured. Next a melting curve was constructed by increasing the temperature from 65 °C to 95 °C in sequential steps of 0.5 °C for 5 s, at which fluorescence was measured. The real-time PCR efficiency of each pair primers was calculated with a 5-point of 5-fold dilution series of cDNA, which was used to construct a relative standard curve. PCR efficiencies ranged between 95% and 105%. A cDNA pool of all samples was used as a calibration and three replications of each sample were performed. The primers for the *FRZB* (KF040089) were the same as those used for semi-quantitative RT-PCR. We selected the housekeeping gene hypoxanthine phospho-ribosyl transferase (*HPRT*) (U69731) as the internal standard (Primers; 5′-CAGTCAACGG GCGATATAAAAGT-3′ and 5′-CCAGTGTCAATTATATCT TCAACAATCA-3′). Gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method

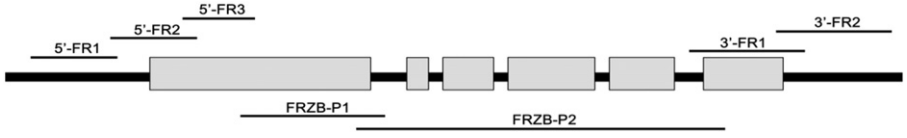

Fig. 1. The pig *FRZB* gene structure and the position of primers used for SNP identification. Note: The black thick lines represent flanking regions and introns; the grey blocks represent exons of *FRZB* gene; the black thin lines represent positions of primer amplicons; The PCR templates of the primers of 5′-FR1, 5′-FR2, 5′-FR3, FRZB-P1, 3′-FR1 and 3′-FR2 are pig total DNA and the PCR templates of the primers of FRZB-P2 are total cDNA of pig muscle tissues.

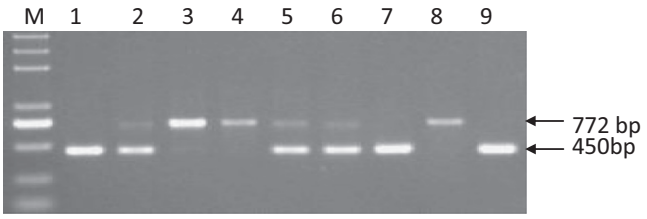

Fig. 2. Electrophoresis of PCR products amplified with the 5′-FR1 primer set (Table 1). Note: Lanes 1, 7 and 9 are genotype AA (no insertion). Lanes 3, 4 and 8 are genotype BB (insertion). Lanes 2, 5 and 6 are genotype AB. M is a DNA marker of DL2000 plus.

($\Delta\Delta Ct = \Delta Ct_{\text{target gene}} - \Delta Ct_{\text{housekeeping gene}}$) as previously described (Livak and Schmittgen, 2001).

2.6. Western blotting

We used the β-Tubulin to quantify pig sFRP3 protein. The *FRZB* antibody was raised against human *FRZB* peptide and the identity was 99% to pig *FRZB* peptide. Proteins (40 μg) were separated by a 5% stacking and a 10% separating sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). After electrophoresis, proteins were transferred to Immobilon-P Transfer Membrane (IPVH00010) at 300 mA for 2 h using a Bio-Rad Criterion Blotter and blocked overnight in blocking buffer (P0023B, Beyotime Ltd., China). Membranes were then incubated with primary mouse monoclonal Anti-β-Tubulin (1:1000, CW0098, CWBIO Ltd., China), or *FRZB* (1:500, ab55026, Abcam, Cambridge, UK) antibodies diluted in primary antibody dilution buffer (P0023A, Beyotime Ltd., China) at 4 °C for 2 h. After three washes with PBST, membranes were incubated with secondary goat anti-mouse-IgG-HRP (1:5000, M21001S) antibody diluted in secondary antibody dilution buffer (P0023D, Beyotime Ltd., China) for 1 h, and washed again with PBST. Immune complexes were visualised using an eECL Western Blot Kit (CW0049A, CWBIO Ltd., China). The expression blots of protein were analysed using ImageJ 1.44 software (NIH, USA) to determine expression ratio of sFRP3 and β-Tubulin.

2.7. Statistical analyses

Gene expression levels in the three pig breeds were analysed by one-way ANOVA with repeated measures using SAS9.1 software (SAS Inst. Inc., Cary, NC). Results were expressed as mean ± standard error. $P < 0.05$ was considered significant. A χ^2 test was used to analyse the distribution of genotypes and to compare differences in genotype distribution.

3. Results

3.1. SNP screening and genotyping

The pig *FRZB* gene structure and the position of primers used for SNP identification were showed in Fig. 1. Three variants were identified in the *FRZB* gene. PCR amplification of a region upstream of the *FRZB* transcription start site (nt (−673)–(−224)) using the 5′-FR1 primers (Table 1) produced an additional amplicon of approximately 772 bp, in addition to the expected 450 bp product (Fig. 2), representing a polymorphic insertion. We designated the no insertion allele 'A' and the insertion variant 'B'. Sequencing demonstrated that the insertion fragment was approximately 330 bp and located −532 nt upstream of the transcription start site. Clear sequence was obtained for both ends of the insertion fragment by paired-end sequencing; however, the central region of the insertion was not sequenced completely due to the presence of a poly T motif on the plus strand (and the complementary poly A of the minus strand). Although the complete sequence of the insertion was not clear, it is still useful as a molecular marker. The genotype distributions of the insertion in DSP, TP, LW, HP, LY, and LL pig populations were shown in Table 2. The polymorphism A-532B was only found in the four indigenous breeds (DSP, TP, LW, and HP) and the genotype distributions were in Hardy–Weinberg Equilibrium ($P > 0.05$).

Two SNPs were detected at 636 and 650 bp of the 5′ flanking region of *FRZB* by sequencing (Fig. 3). No SNPs were detected in the coding or 3′-UTR regions of *FRZB*. The genotype and allele frequencies of the two SNPs in the different breeds were listed in Table 2. All three genotypes of both SNPs were represented in all six populations. The G636A genotypes were in Hardy–Weinberg Equilibrium ($P > 0.05$) among LW, HP, LY, and LL populations, with frequencies of allele A higher in the indigenous breeds (0.47–0.95) than in introduced pigs (0.38–0.41). C650T genotypes were only in Hardy–Weinberg Equilibrium ($P > 0.05$) among TP, HP, and LL populations. The genotype TT was dominant in the mini-type indigenous breeds (DSP and TP; frequencies of 0.83 and 0.50, respectively) and the CC genotype was more common among

Table 2
Gene and genotype frequency of the three SNPs in different pig breeds.

Loci	Breed	Genotype frequency (number/frequency)				Allele frequency	
		AA	AB	BB	HWE χ^2 value (P value)	A	B
A-532B	DSP	18/0.3051	27/0.4577	14/0.2372	0.3825 (P = 0.5363)	0.5339	0.4661
	TP	66/0.8571	11/0.1429	0/0.0000	0.4556 (P = 0.4997)	0.9286	0.0714
	LW	25/0.4902	25/0.4902	1/0.0196	3.4280 (P = 0.0641)	0.7353	0.2647
	HP	23/0.8846	3/0.1154	0/0.0000	0.0975 (P = 0.7549)	0.9423	0.0577
	LY	91/1.0000	0/0.0000	0/0.0000	–	1	0
	LL	48/1.0000	0/0.0000	0/0.0000	–	1	0
		GG	GA	AA	HWE χ^2 value (P value)	G	A
G636A	DSP	1/0.0333	1/0.0333	28/0.9333	12.6408 (P = 0.0004)	0.0500	0.9500
	TP	2/0.0667	1/0.0333	27/0.9000	18.3372 (P = 1.85E−05)	0.0833	0.9167
	LW	5/0.1724	10/0.3448	14/0.4828	1.6267 (P = 0.2022)	0.3448	0.6552
	HP	9/0.3000	14/0.4667	7/0.2333	0.1172 (P = 0.7321)	0.5333	0.4667
	LY	17/0.3541	23/0.4792	8/0.667	0.0022 (P = 0.9627)	0.5938	0.4062
	LL	10/0.3448	16/0.5517	3/0.1034	0.8551 (P = 0.3551)	0.6207	0.3793
		CC	CT	TT	HWE χ^2 value (P value)	C	T
C650T	DSP	2/0.0667	3/0.1000	25/0.8333	7.9513 (P = 0.004)	0.1167	0.8833
	TP	2/0.0667	13/0.4333	15/0.5000	0.3704 (P = 0.5428)	0.2833	0.7167
	LW	16/0.5517	4/0.137	9/0.3103	14.4985 (P = 0.0001)	0.6207	0.3793
	HP	9/0.3000	14/0.4667	7/0.2333	0.1172 (P = 0.7321)	0.5333	0.4667
	LY	46/0.9583	1/0.0208	1/0.0208	20.6507 (P = 5.51E−06)	0.9688	0.0312
	LL	26/0.8966	3/0.1034	0/0.0000	0.0863 (P = 0.7690)	0.9483	0.0517

For locus A-532B allele A has no insertion, and allele B has the insertion. HWE = Hardy–Weinberg Equilibrium.

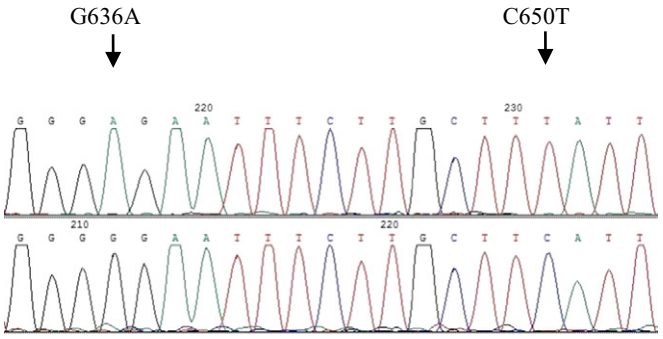

Fig. 3. The sequencing chromatogram in the sites of two SNPs of G636A and C650T. Note: Chromatogram illustrating the sequence of the PCR product amplified using the 5′-FR3 primer set (Table 1) shows the two identified SNPs of G636A and C650T. The G636A refers to a mutation of G to A at the 636 bp and the C650T refers to a mutation of C to T at the 650 bp of the transcription start site of the *FRZB* gene.

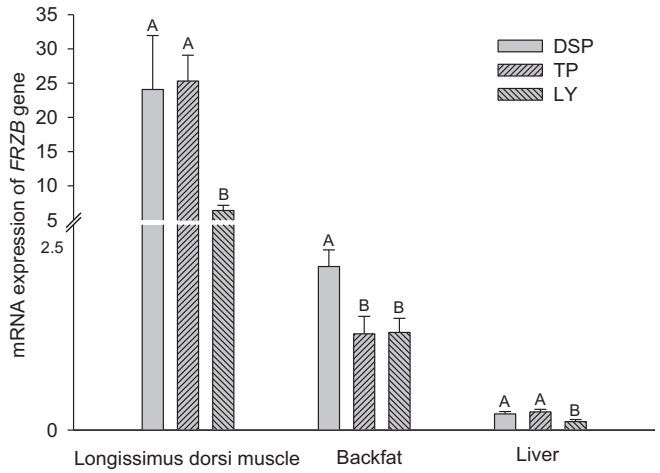

Fig. 5. *FRZB* mRNA expression levels in the tissues of LD, back fat and liver of the three pig breeds. Note: The mRNA expression values are calculated by ration of test samples to a calibration sample. Break range on Y axis is 2.5–5 being omitted. Error bars represent SE of expression. Capital letters on the bars denote the difference of expression level with significant difference, differing letters between groups at the same tissue indicate significant differences ($P < 0.05$), and the same letters indicate no significant differences. DSP = Diannan Small-ear pig (n = 8). TP = Tibetan pig (n = 8). LY = Large Yorkshire (n = 8).

introduced breeds (LY and LL; frequencies of 0.96 and 0.90, respectively), with the three genotypes relatively balanced in the normal indigenous breeds (LW and HP) (Table 2).

3.2. mRNA expression

FRZB transcripts were detected in the heart, liver, lung, kidney, back fat, belly fat, LD, hypothalamus, hypophysis, and brain tissues of the TP (Fig. 4). Among these tissues, expression of the *FRZB* gene was abundant in hypophysis, LD, back fat, and belly fat, modest in liver, lung, and kidney, and minor in heart, hypothalamus, and brain. Indicating that *FRZB* expression is high in tissues involved in growth and fat deposition, including the hypophysis, LD, back fat, and belly fat.

FRZB mRNA expression levels differed among three breeds and three tissues (Fig. 5), with higher expression in LD than in back fat or liver, consistent with the results above. In LD and liver tissues, there was no significant difference between the two indigenous breeds, DSP and TP ($P > 0.05$); however, these strains had significantly higher expression than the introduced breed, LY ($P < 0.05$). In back fat, expression was significantly higher in DSP than in TP or LY ($P < 0.05$), with no significant difference between TP and LY ($P > 0.05$).

3.3. Protein expression

The expression abundance of sFRP3 protein encoded by the *FRZB* gene was different among the three breeds (Fig. 6B), consistent with mRNA expression of the *FRZB* gene in LD, back fat, and liver. In *longissimus dorsi* muscle and liver, sFRP3 protein levels were higher in DSP and TP than in LY (Fig. 6A). However, in back fat sFRP3 protein levels were higher in DSP than in TP and LY (Fig. 6A).

4. Discussion

FRZB is a member of the Wnt signalling pathway, and has a key feature of abundant cysteine in its extracellular glycoprotein domains, which can influence normal cellular processes through provoking frizzled receptors (Moon et al., 2004). The sFRP family consists of at least five proteins, which antagonise the Wnt signalling pathway through their effects on frizzled receptors (Kawano and Kypta, 2003). *FRZB*/sFRP3 also has a role in mammalian skeleton development (Hoang et al., 1996), regulating cell growth and proliferation (Kongkham et al., 2010), and positively regulates adipocyte differentiation (Bennett et al., 2002). Therefore, pig *FRZB* was selected from genes identified in our RNA-seq experiment as a candidate gene for growth and fat deposition traits.

One insertion mutation, located at −532 bp of upstream of the *FRZB* transcription start site, and two SNPs 636 bp and 650 bp of the 5′ flanking region of the *FRZB* gene, were identified in the different breeds. Although the insertion only appeared in the Chinese indigenous breeds, the frequency varied between the two mini-type breeds (DSP and TP) and the two normal indigenous breeds (LW and HP); the insertion mutation might be not associated with pig growth traits. The A allele of G636A and the T allele of C650T were found at higher frequency in the two mini-type breeds (DSP and TP), and at modest frequency in the two indigenous breeds (LW and HP), and lower in the two introduced breeds (LY and LL), indicating that these two SNPs may be associated with growth traits. Further association analysis between the SNPs and growth traits is required in different populations.

For the two SNPs in the 5′-UTR, transcription factor binding sites were predicted using the online software CONSITE (http://asp.ii.uib.no:8090/cgi-bin/consite/consite). Binding sites for Dorsal_2, P50, and P65 transcription factors were disrupted when the G was mutated to an A at the G 636A site, and the binding site for Pax6 was removed and recognition motifs for HMG-IY, TBP, Broad-complex_4, and MEF2 were created when the C was mutated to T at the C650T site, indicating that the SNPs are potential regulatory sites influencing the expression of the *FRZB* gene.

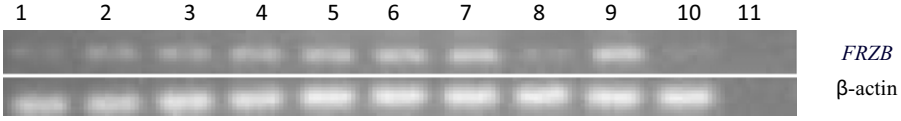

Fig. 4. The expression abundance of *FRZB* mRNA in Tibetan pig tissues. Note: 1, heart; 2, liver; 3, lung; 4, kidney; 5, back fat; 6, belly fat; 7, *longissimus dorsi* muscle (LD); 8, hypothalamus; 9, hypophysis; 10, brain; and 11, negative control. Among these tissues, expression of *FRZB* gene was abundant in hypophysis, LD, back fat, and belly fat, modest in liver, lung, and kidney, and minor in heart, hypothalamus, and brain.

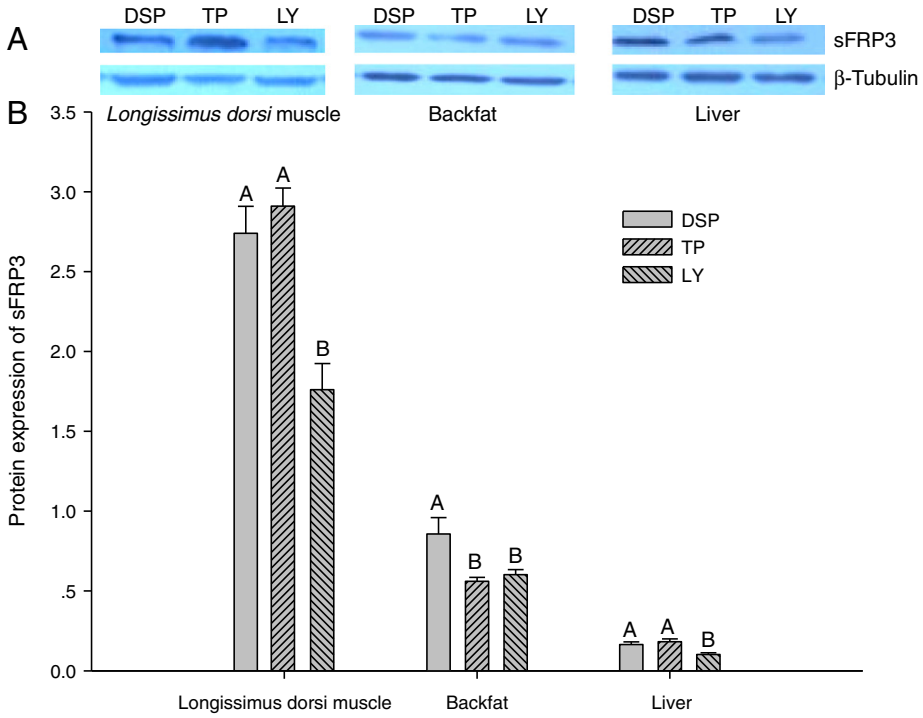

Fig. 6. sFRP3 protein expression levels in three tissues of the three pig breeds. Note: Panel A shows the blots of sFRP3 protein in tissues of *longissimus dorsi* muscle, back fat and liver. Panel B shows the protein expression value calculated by ratio of grey value of sFRP3 to β-Tubulin on the blot membranes. Error bars represent SE of the expression. Capital letters on the bars denote the difference of expression level with significant difference, differing letters between groups at the same tissue indicate significant differences ($P < 0.05$), and the same letters indicate no significant differences. DSP = Diannan Small-ear pig (n = 8). TP = Tibetan pig (n = 8). LY = Large Yorkshire (n = 8).

Determination of *FRZB* mRNA abundance in ten tissues indicated that expression was highest in hypophysis, LD, and adipose tissue, which are important parts of the growth axis in mammals, and in the back fat and belly fat, which directly affect the lean ratio of pigs. High expression in these tissues demonstrates that the *FRZB* gene is associated with growth and fat deposition traits in pigs.

Up to now, we can get few of reports about pig *FRZB* from PubMed or other Databases. Human sFRP3 as a Wnt pathway antagonist, reduces activity of metalloproteinases and activation of β-catenin and can inhibit epithelial–mesenchymal transition (EMT) seen in several cancer types (Pecina-Slaus et al., 2010; Zi et al., 2005). It was reported that the sFRP family had the function of reducing expression in several types of carcinomas, which has a relationship with unfavourable clinical results (Shi et al., 2007). The results from previous studies suggested the low expression of *FRZB* in tumour tissues and then proved the function of *FRZB*/sFRP3 in suppression of tumour activities (Nikuseva-Martic et al., 2013). Through comparing the expression of sFRP3 in primary cancer tissues with that in normal kidney tissues, it also indicated the sFRP3 does decrease in tissues related to tumour (Hirata et al., 2010). These findings above provide evidence that decreased expression of the *FRZB* is favourable to the growth of tumours, which shows that *FRZB* can restrain the activity of growth. Real-time PCR and western blotting demonstrated that *FRZB*/sFRP3 expression levels in the LD and liver of slow-growth breeds were higher than those in fast-growth pigs, indicating that *FRZB* might negatively regulate the growth rate of muscles. However, in back fat tissues, expression levels were higher in DSP than in TP and LY, indicating that *FRZB*/sFRP3 might positively regulate fat deposition.

In conclusion, pig *FRZB* shows differential expression in pig tissues and among different breeds, and may play a key role in negative regulation of growth rate and positive regulation of fat deposition.

Conflict of interest

The authors have no conflict of interest each other.

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