Original Article

Molecular Characterization, Polymorphism and Association of Porcine *IBP4* Gene

(*IBP4* / litter size / pig / polymorphism / RT-PCR)

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Abstract. The complete coding sequence of the porcine *IBP4* gene was isolated using RT-PCR. Sequence analysis showed that the porcine *IBP4* gene encodes a protein of 259 amino acids which shares high homology with the insulin-like growth factor binding protein 4 (IBP4) of eight species: cattle (97 %), goat (97 %), chimpanzee (97 %), human (96 %), giant panda (96 %), sheep (95 %), Sumatran orangutan (95 %) and rabbit (93 %). This gene is structured in four exons and three introns as revealed by computer-assisted analysis. Phylogenetic analysis revealed that the porcine *IBP4* gene has a close genetic relationship with the *IBP4* gene of cattle. Polymorphism analysis indicated that there was an A/G substitution in the position of 134 bp of exon 2 and this mutation did not alter the encoded amino acids of the porcine *IBP4* gene. PCR-*Hha* I-RFLP revealed that eight pig breeds displayed obvious genotype and allele frequency differences at this polymorphic locus. Association of this SNP with litter size traits was assessed in Large White (N = 100) and Landrace (N = 100) populations, and the results demonstrated that this polymorphic locus was significantly associated with the litter size of the first parity in Large White sows and Landrace sows (P <0 .05). These data serve as a foundation for further insight into this novel porcine gene.

Introduction

Insulin-like growth factor binding protein 4 (IBP4) is a member of the insulin-like growth factor binding protein (IGFBP) family. This protein has an IGFBP domain and a thyroglobulin type-I domain. It binds both insulin-like growth factors (IGFs) I and II and circulates in the plasma in both glycosylated and non-glycosylated forms. Binding of this protein prolongs the half-life of the IGFs and alters their interaction with cell surface receptors (Canzian et al., 2010; Giroux et al., 2010; Gu et al., 2010; He et al., 2010; Sato et al., 2011). However, latest studies have shown that the *IBP4* gene is also an important reproduction-related gene for it has been identified to be associated with oocyte maturation and embryo development (Qin et al., 2002; Carter et al., 2005; Wang et al., 2006).

As mentioned above, the *IBP4* gene is an important gene with many biological functions. Until today, the *IBP4* gene has been reported in human, rabbit, cattle and other animals, but the porcine *IBP4* gene has not been reported yet.

In the present experiment, we cloned the full-length cDNA sequence of the porcine *IBP4* gene, and further performed the necessary sequence and polymorphic analysis. Furthermore, we examined the porcine *IBP4* gene as a candidate gene for porcine reproductive traits through association analysis with the litter size.

Material and Methods

Animals and Sample Preparation

Six adult Large White pigs were slaughtered. Large intestine, spleen, lung, muscle, fat, liver, heart, kidney

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Abbreviations: BAC – bacterial artificial chromosome, EST – expressed sequence tag, GLM – general linear model, *IBP4* – insulin-like growth factor binding protein 4 gene, IGF – insulin-like growth factor, IGFBP – insulin-like growth factor binding protein, MAS – marker assistant selection, NBA – number of piglets born alive, ORF – open reading frame, PCR – polymerase chain reaction, RFLP – restriction fragment length polymorphism, RT-PCR – reverse transcriptase-polymerase chain reaction, TNB – total number of piglets born.

Table 1. The information on 795 unrelated pigs from eight populations

Breed	Sampling location	Sample size		
		Total	Male	Female
Large White pig	Guangdong Province	100	0	100
Landrace pig	Guangdong Province	100	0	100
Saba pig	Dongchuan county of Yunnan Province	100	50	50
Tibetan pig	Xianggelila county of Yunnan Province	95	50	45
Mingguang small-ear pig	Tengchong county of Yunnan Province	100	50	50
Diannan small-ear pig	Banna state of Yunnan Province	100	50	50
Wujin pig	Qujing city of Yunnan Province	100	50	50
Baoshan pig	Baoshan city of Yunnan Province	100	50	50

and ovary samples were collected, frozen in liquid nitrogen, and then stored at –80 °C. Total RNA was extracted using the Total RNA Extraction Kit (Gibco, Life Technologies, Grand Island, NY). RNA samples were pooled and used to perform RT-PCR for cloning the coding sequence of the porcine *IBP4* gene. RNA reverse transcription and first-strand cDNA synthesis were conducted as previously described (Liu et al., 2004).

Ear samples were collected from 795 unrelated animals belonging to eight porcine populations presented in Table 1. Genomic DNA isolated from these ear samples were used to perform the polymorphism analysis.

Both the total number of piglets born (TNB) and the number of piglets born alive (NBA) of 100 Large White sows and 100 Landrace sows listed in Table 1 were recorded for 700 litters. The litter size traits data and genomic DNA of these pigs were used to perform association analysis.

Isolation of the coding sequences for the porcine IBP4 gene

The RT-PCR was performed to isolate the coding sequence for the porcine *IBP4* gene using the pooled cDNA from different tissues mentioned above. The primers for porcine *IBP4* gene isolation were designed based on the coding sequence of the human *IBP4* gene and their highly homologous pig EST sequences: FS675354, BP151209 and the PCR primers were forward primer 1 and reverse primer 1 (listed in Table 2). The 25 µl reaction system was: 2.0 µl cDNA (100 ng/µl), 2.5 µl 2 mM mixed dNTPs, 2.5 µl 10× Taq DNA polymerase buffer, 2.5 µl 25 mM $MgCl_2$, 2.0 µl 10 µM forward primer 1, 2.0 µl 10 µM reverse primer 1, 2.0 units of Taq DNA polymerase (1 U/1 µl), and 9.5 µl sterile water. The PCR programme initially started with a 94 °C denaturation for 4 min, followed by 35 cycles of 94 °C/50 s, 62 °C/50 s, 72 °C/50 s, then 72 °C extension for 10 min, finally 4 °C to terminate the reaction.

The PCR products were then cloned into the pMD18-T vector (TaKaRa, Dalian, China) and sequenced bi-directionally by the commercial fluorometric method (SHENGGONG, Shanghai, China). At least five independent clones were sequenced for each PCR product.

Sequence analysis

Gene analysis for the cDNA sequence was conducted using GenScan software (http://genes.mit.edu/GENSCAN.html). The protein prediction and analysis were performed using the Conserved Domain Architecture Retrieval Tool of BLAST at the National Center for Biotechnology Information (NCBI) server (http://www.ncbi.nlm.nih.gov/BLAST) and the ClustalW software (http://align.genome.jp/). The theoretical isoelectric point (pI) and molecular weight (Mw) of proteins was computed using the Compute pI/Mw Tool (http://www.expasy.org/tools/pi_tool.html).

PCR-RFLP

The DNA from the above-mentioned pigs (Table 1) was used as a template to perform PCR with forward primer 2 and reverse primer 2 (listed in Table 2). The 25 µl reaction system was: 2.0 µl DNA (100 ng), 2.5 µl 2 mM mixed dNTPs, 2.5 µl 10× Taq DNA polymerase buffer, 2.5 µl 25 mM $MgCl_2$, 1.0 µl 20 µM forward primer 2, 1.0 µl 20 µM reverse primer 2, 1.0 units of Taq DNA polymerase (1 U/µl) (Jinmei Biotech Corporation, Tianjin, China), and 12.5 µl sterile water. PCR was run as follows: 94 °C for 4 min, followed by 35 cycles of 94 °C for 50 s, 55 °C for 50 s, 72 °C for 1 min, then 72 °C extension for 10 min, finally 4 °C to terminate the reaction.

The PCR products were then cloned into the pMD18-T vector (TaKaRa) and sequenced bi-directionally by the commercial fluorometric method (SHENGGONG). At least five independent clones were sequenced for each PCR product.

Table 2. Primers for the porcine IBP4 gene and their annealing temperatures

Gene	Primer sequences	Region	Position	Product length (bp)	Tm (°C)
IBP4	Forward primer 1 : 5'- ATGCTGCCCCTGTGCCTC -3'	Exon 1	1–18	780	62
	Reverse primer 1: 5'- TCACTCTCGGAAGCTGTCG -3'	Exon 4	117–135		
	Forward primer 2: 5'- TCCATTCACCCTGCTCAT -3'	Intron 1	7524–7541	320	55
	Reverse primer 2: 5'- TGCACATGCGCTTTTGGT -3'	Intron 2	12–29		

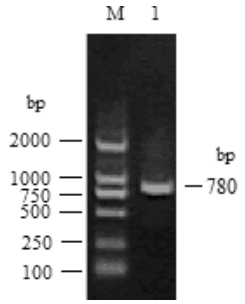

Fig. 1. RT-PCR result for the porcine *IBP4* gene. M: DL2000 DNA markers; 1: PCR product for the porcine *IBP4* gene.

The 31 µl PCR-RFLP reaction volume consisted of: 10 µl PCR product, 18 µl sterile water, 1 µl *Hha* I (10 U), 2 µl 10× buffer. The mixture was incubated in an air incubator at 37 °C for 4 h, and then the genotypes were analysed in agarose gel (2.5%) containing ethidium bromide.

Statistical analysis

The relationships between *IBP4* genotypes and litter size traits of Large White (N = 100) and Landrace (N = 100) sows were evaluated with the general linear model (GLM) procedure of SAS version 8.0. Both additive and dominance effects were also estimated using the REG procedure, where the additive effect was estimated as -1, 0 and 1 for the AA, CA and CC genotype, respectively; and the dominance effect represented as 1, -1 and 1 for the AA, CA and CC genotype, respectively (Zhang et al., 2009). The model: $Yijkl = \mu + Pi + Sj + Fk + Gl + eijkl$, where *Yijkl* is the observation of the trait, μ is the least square means, *Pi* is the effect of i^{th} parity (i =1, 2, 3, 4,5,6,7 (parity ≥ 7)), *Sj* is the effect of j^{th} season, *Fk* is the effect of k^{th} farm (k=1, 2), *Gl* is the effect of l^{th} genotype (l = 1–3) and *eijkl* is the random residual (Niu et al., 2009).

Results

Isolation of the coding sequences for the porcine IBP4 gene

For the porcine *IBP4* gene, one PCR product of 780-bp was obtained using RT-PCR (Fig. 1).

Sequence analysis

The cDNA nucleotide sequence analysis using the BLAST software revealed that this gene was not homologous to any of the known pig genes and it was then deposited into the GenBank database (Accession number: DQ917619). The sequence prediction was carried out using the GenScan software and the results showed that this 780-bp cDNA sequence represented one single gene which encoded 259 amino acids (Fig. 2). The theoretical isoelectric point (pI) and molecular weight (Mw) of this deduced protein were computed using the Compute pI/Mw Tool. The pI of pig IBP4 is 7.79. The molecular weight of this putative protein is 28230.43.

BLAST analysis also revealed that the pig IBP4 protein shares high homology with the insulin-like growth factor binding protein 4 (IBP4) of eight species: cattle (accession number: NP_776982; 97 %), goat (accession number: ACB45432; 97 %), chimpanzee (accession number: XP_511475; 97 %), human (accession number: NP_001543; 96 %), giant panda (accession number: XP_002924982; 96 %), sheep (accession number: NP_001127774; 95 %), Sumatran orangutan (accession number: XP_002827645; 95 %) and rabbit (accession number: XP_002719403; 93 %) (Fig. 3).

Based on the results of the alignment of IBP4 proteins, a phylogenetic tree was constructed using the Dendrogram procedure of ClustalW software (http://align.genome.jp/), as shown in Fig. 4.

The phylogenetic analysis revealed that the porcine *IBP4* gene has a close genetic relationship with the *IBP4* gene of cattle.

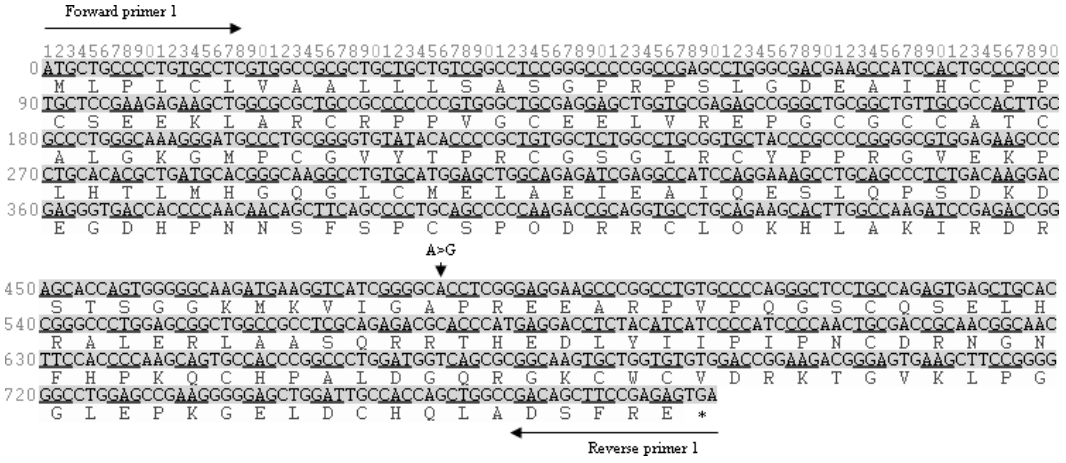

Fig. 2. The cDNA and amino acid sequence of the porcine *IBP4* gene (GenBank accession number: DQ917619). ATG, start codon; TAG, stop codon (* – stop codon). The arrows indicate the positions of mutation (A>G) and primers of RT-PCR (Forward primer1 and Reverse primer1)

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Sumatran orangutan      MLPLCLVAALMLAA-GPGPNLGDEAIHCPPCSEEKLARCRPPVGCEELVREPGCGCCATC 59
Human                   MLPLCLVAALLLAA-GPGPSLGDEAIHCPPCSEEKLARCRPPVGCEELVREPGCGCCATC 59
Chimpanzee              MLPLCLVAALLLAA-GPGPSLGDEAIHCPPCSEEKLARCRPPVGCEELVREPGCGCCATC 59
Sheep                   MLSLCLVTALLLAA-GPGPSLGDEAIHCPPCSEEKLARCRPPVGCEELVREPGCGCCATC 59
Goat                    MLSLCLVAALLLAA-GPGPSLGDEAIHCPPCSEEKLARCRPPVGCEELVREPGCGCCATC 59
Cattle                  MLSLCLMAALLLAA-GPGPSLGDEAIHCPPCSEEKLARCRPPVGCEELVREPGCGCCATC 59
Pig                     MLPLCLVAALLLSASGPRPSLGDEAIHCPPCSEEKLARCRPPVGCEELVREPGCGCCATC 60
Giant panda             MLPLCLVAALLLAA-GPGPSLGDEAIHCPPCSEEKLARCRPPVGCEELVREPGCGCCATC 59
Rabbit                  MLSLCLVVALLLAS-GPQPSLGDEAIHCPPCSEEKLARCRPPVGCEELVREPGCGCCATC 59
                        **.***:.**:*:: ** *.****************************************

Sumatran orangutan      ALGLGMPCGVYTPRCGSGLRCYPPRGVEKPLHTLMHGQGVCMELAEIEAIQESLQPSDKD 119
Human                   ALGLGMPCGVYTPRCGSGLRCYPPRGVEKPLHTLMHGQGVCMELAEIEAIQESLQPSDKD 119
Chimpanzee              ALGLGMPCGVYTPRCGSGLRCYPPRGVEKPLHTLMHGQGLCMELAEIEAIQESLQPSDKD 119
Sheep                   ALGKGMPCGVYTPRCGSGLRCYPPRGVEKPLHTLVHGQGVCMELAEIEAIQESLQPSDKD 119
Goat                    ALGKGMPCGVYTPRCGSGLRCYPPRGVEKPLHTLVHGQGVCMELAEIEAIQESLQPSDKD 119
Cattle                  ALGKGMPCGVYTPRCGSGLRCYPPRGVEKPLHTLVHGQGVCMELAEIEAIQESLQPSDKD 119
Pig                     ALGKGMPCGVYTPRCGSGLRCYPPRGVEKPLHTLMHGQGLCMELAEIEAIQESLQPSDKD 120
Giant panda             ALGKGMPCGVYTPRCGSGLRCYPPRGVEKPLHTLMHGQGVCMELAEIEAIQESLQPSDKD 119
Rabbit                  ALGLGMPCGVYTQRCGSGLRCYPPRGVEKPLHTLMQGQGVCMELAEIEAIQASMQPSDKD 119
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Sumatran orangutan      EGDHPNNSFSPCSAHDRRCLQKHFAKIRDRSTSGGKMKVNGAPREDARPVPQGSCQSELH 179
Human                   EGDHPNNSFSPCSAHDRRCLQKHFAKIRDRSTSGGKMKVNGAPREDARPVPQGSCQSELH 179
Chimpanzee              EGDHPNNSFSPCSAHDRRCLQKHFAKIRDRSTSGGKMKVNGAPREDARPVPQGSCQSELH 179
Sheep                   EGDHPNNSFSPCSAHDRKCLQKHLAKIRDRSTSGGKMKVIGAPREEVRPVPQGSCQSELH 179
Goat                    EGDHPNNSFSPCSAHDRKCLQKHLAKIRDRSTSGGKMKVIGAPREEVRPVPQGSCQSELH 179
Cattle                  EGDHPNNSFSPCSAHDRKCLQKHLAKIRDRSTSGGKMKVIGAPREEARPVPQGSCQSELH 179
Pig                     EGDHPNNSFSPCSPQDRRCLQKHLAKIRDRSTSGGKMKVIGAPREEARPVPQGSCQSELH 180
Giant panda             EGEHPNNSFSPCSAHDRRCLQKHFAKIRDRSSSGGKMKVIGVPREEARPVPQGSCQSELH 179
Rabbit                  EGDHPNNSFSPCSAHDRRCLQKHFAKMRDRSSSGGKMKIIGAPREDVRPVPQGSCQSELH 179
                        **:**********.:**:*****:**:****:******: *.***:.************

Sumatran orangutan      RALERLAASQSRTHEDLYIIPIPNCDRNGNFHPKQCHPALDGQRGKCWCVDRKTGVKLPG 239
Human                   RALERLAASQSRTHEDLYIIPIPNCDRNGNFHPKQCHPALDGQRGKCWCVDRKTGVKLPG 239
Chimpanzee              RALERLAASQSRTHEDLYIIPIPNCDRNGNFHPKQCHPALDGQRGKCWCVDRKTGVKLPG 239
Sheep                   RALERLAASQSRTHEDLYIIPIPNCDRNGNFHPKQCHPALDGQRGKCWCVDRKTGVKLPG 239
Goat                    RALERLAASQSRTHEDLYIIPIPNCDRNGNFHPKQCHPALDGQRGKCWCVDRKTGVKLPG 239
Cattle                  RALERLAASQSRTHEDLYIIPIPNCDRNGNFHPKQCHPALDGQRGKCWCVDRKTGVKLPG 239
Pig                     RALERLAASQRRTHEDLYIIPIPNCDRNGNFHPKQCHPALDGQRGKCWCVDRKTGVKLPG 240
Giant panda             RALERLAASQSRTHEDLYTIPIPNCDRNGNFHPKQCHPALDGQRGKCWCVDRKTGVKLPG 239
Rabbit                  RALERLAASQSRTHEDLFIIPIPNCDRNGNFHPKQCHPALDGQRGKCWCVDRKTGVKLPG 239
                        ********** ******: ****************************************

Sumatran orangutan      GLEPKGELDCHQLADSFRE 258
Human                   GLEPKGELDCHQLADSFRE 258
Chimpanzee              GLEPKGELDCHQLADSFRE 258
Sheep                   GLEPKGELDCHQLADSFRE 258
Goat                    GLEPKGELDCHQLADSFRE 258
Cattle                  GLEPKGELDCHQLADSFRE 258
Pig                     GLEPKGELDCHQLADSFRE 259
Giant panda             GLEPKGELDCHQLADSFRE 258
Rabbit                  GLEPKGELDCHQLADSFRE 258
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Fig. 3. The alignment of the protein encoded by the porcine *IBP4* gene and other eight kinds of IBP4 proteins

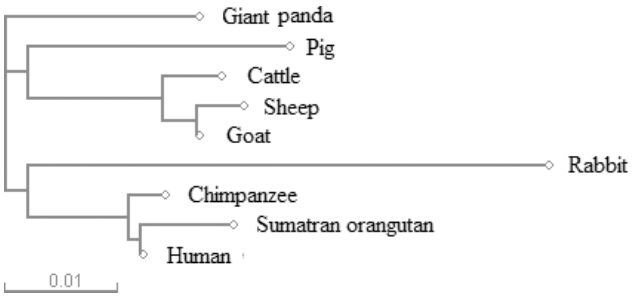

Fig. 4. The phylogenetic analysis for the *IBP4* gene

To obtain the genomic DNA of the *IBP4* gene, the publicly available pig genome database at the NCBI Pig Genome Resources (http://www.ncbi.nlm.nih.gov/projects/genome/guide/pig/) was screened using the cDNA sequence of the *IBP4* gene as a seed. A bacterial artificial chromosome (BAC) clone (Sus scrofa chromosome 12 clone CH242-522D8, GenBank accession no. CU928542), which encompasses the entire *IBP4* gene, was identified by BLASTGen analysis. The porcine *IBP4* gene (nucleotides 31,918–45,394 in the Sus scrofa chromosome 12 clone CH242-522D8) is 13,477 bp in length and consists of four exons. All exon-intron splice junction sequences conform to the GT-AG rule (Fig. 5).

Polymorphism

Comparing the cDNA and DNA sequences of the porcine *IBP4* gene, one A/G mutation was found in the position of 134 bp of exon 2. Based on the DNA sequence of the porcine *IBP4* gene, primers (forward primer 2 and reverse primer 2) were designed and used to amplify the

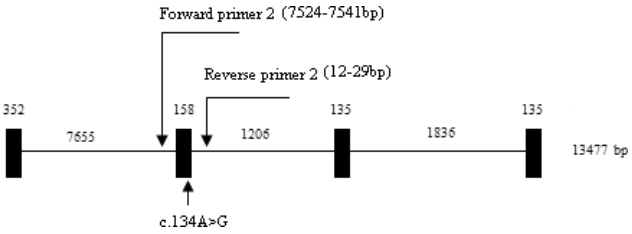

Fig. 5. The genomic sequence organization representing the ORF of the porcine *IBP4* gene. The arrows indicate the positions of mutation (A>G) and primers for PCR-RFLP (forward primer 2 and reverse primer 2).

DNA of Large white and Landrace. The PCR products were then cloned into the PMD18-T vector and sequenced bi-directionally by the commercial fluorometric method. At least five independent clones were sequenced for each PCR product. Through sequencing, this A/G mutation was confirmed in the position of 134 bp of exon 2. This substitution led to mutation of one *Hha* I restriction site and did not alter the encoded amino acids. This was also confirmed by PCR-*Hha* I-RFLP (Fig. 6).

Subsequently, PCR-*Hha* I-RFLP were performed using the DNA from 795 unrelated animals belonging to eight pig populations including Large White pig, Landrace pig, Saba pig, Tibetan pig, Mingguang small-ear pig, Diannan small-ear pig, Wujin pig and Baoshan pig. The AA and GA genotypes had not been detected in any of the six Yunnan local pig breeds. Only a small number of animals of these two genotypes were found among Large White pigs and Landrace pigs. The results revealed that the frequency of the A allele in the two European pig breeds: Large White pig (0.090) and Landrace pig (0.065) was higher than that in other six Yunnan local pig breeds: Saba pig (0), Tibetan pig (0), Mingguang small-ear pig (0), Diannan small-ear pig (0), Wujin pig (0) and Baoshan pig (0). The two exotic pig breeds: Large White pig and Landrace pig had more animals of genotypes GA and AA. This indicated that Yunnan local pig breeds and European pig breeds displayed obvious genotype and allele frequency differences at this A/G mutation locus.

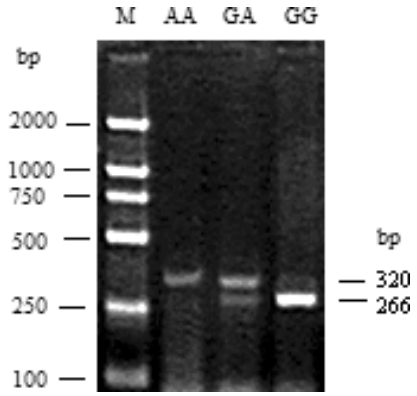

Fig. 6. Polymorphism analysis of the porcine *IBP4* gene by PCR-*Hha* I-RFLP.
M: DL2000 DNA markers; AA*:* 320 bp; GA: 320 bp + 266 bp + 54 bp; GG: 266 bp + 54 bp

Association of the SNP and litter size was assessed in two populations (purebred Large White and purebred Landrace sows). Statistical analysis demonstrated that, for the litter size of all parities, no significant difference was found both in the experimental purebred Large White sows and in the experimental purebred Landrace sows. For the litter size of the first parity, in the purebred Large White sows, those with the AA genotype had an additional 1.337 piglets born and an additional 0.946 piglets born alive compared to the GG animals ($P < 0.05$). In addition, for the first parity, in the purebred Landrace sows, AA animals had 0.835 more piglets born than the GG animals ($P < 0.05$).

Discussion

In the current study, we firstly obtained the full-length coding sequence of the porcine *IBP4* gene. Through sequence analysis, it can be seen that the protein encoding the porcine *IBP4* gene is highly homologous with IBP4 proteins of human, cattle and other mammals. This implies that the *IBP4* genes were highly conserved in some mammals and the porcine *IBP4* gene might have similar functions as the *IBP4* genes of human, cattle and other mammals. It can also be found that the porcine IBP4 protein does not show complete identity to human, cattle or other mammals. This suggests that the porcine *IBP4* gene might have some differences in functions to those of human, cattle or other mammals. In phylogenetic analysis we found that the porcine *IBP4* gene has a close genetic relationship with the *IBP4* gene of cattle, and this implied that we could use cattle as a model organism to study the porcine *IBP4* gene.

Table 3. Allele frequency and genotype of Hha I polymorphic locus in different pig breeds

Breed	Number of pigs	Genotype			Allele frequency	
		GG	GA	AA	G	A
Large White pig	100	88	6	6	0.910	0.090
Landrace pig	100	90	7	3	0.935	0.065
Saba pig	100	100	0	0	1.00	0
Tibetan pig	95	95	0	0	1.00	0
Mingguang small-ear pig	100	100	0	0	1.00	0
Diannan small-ear pig	100	100	0	0	1.00	0
Wujin pig	100	100	0	0	1.00	0
Baoshan pig	100	100	0	0	1.00	0

Table 4. Association between porcine IBP4 gene PCR-Hha I-RFLP genotypes and litter size traits

Breed	Traits		Genotype (mean ± S.E.) GG	GA	AA	Genetic effects (mean ± S.E.) Additive	Dominant
Large white	1st parity	N	88	6	6		
		TNB	10.500 ± 0.401[a]	10.731 ± 0.32629	11.837 ± 0.395[b]	0.668 ± 0.181	-0.437 ± 0.213
		NBA	8.658 ± 0.2176[a]	8.812 ± 0.434	9.604 ± 0.390[b]	0.473 ± 0.176	-0.319 ± 0.210
	All parities	N	88	6	6		
		TNB	12.073 ± 0.391	12.093 ± 0.241	12.209 ± 0.325	0.068 ± 0.176	-0.048 ± 0.130
		NBA	10.292 ± 0.327	10.325 ± 0.521	10.531 ± 0.224	0.119 ± 0.209	-0.086 ± 0.134
Landrace	1st parity	N	90	7	3		
		TNB	10.94 7± 0.375[a]	11.319 ± 0.378	11.782 ± 0.678[b]	0.417 ± 0.215	-0.045 ± 0.128
		NBA	9.573 ± 0.268	9.595 ± 0.419	9.758 ± 0.281	0.092 ± 0.198	-0.070 ± 0.265
	All parities	N	90	7	3		
		TNB	12.250 ± 0.507	12.704 ± 0.486	12.864 ± 0.371	0.307 ± 0.246	0.147 ± 0.150
		NBA	10.275 ± 0.597	10.307 ± 0.272	10.432 ± 0.337	0.078 ± 0.189	-0.046 ± 0.147

N: Number of investigated litters. Least square mean values with different letters are significantly different: small letter – $P < 0.05$.

The involvement of the *IBP4* gene in the reproduction process was a molecular basis for association analysis of this gene DNA polymorphism with litter size traits of the pig (Qin et al., 2002; Carter et al., 2005; Wang et al., 2006). Based on association analysis of the SNP and litter size, it could be found that the polymorphism (A>G) of the porcine *IBP4* gene can significantly affect litter size. The AA genotype animals obviously have better litter size of the first parity than the GA and GG animals both in purebred Large White and purebred Landrace sows. This indicates that this polymorphic locus of the porcine *IBP4* gene is a valuable marker deserving to be applied to the marker assistant selection (MAS) in pig breeding. Therefore, the *IBP4* gene could be a useful candidate gene in selection for increasing litter size in pigs. Pig industry can select and maintain more AA animals to improve the reproductive performance of sows in pig production. We also noticed that only small numbers of GA and AA genotype animals had been found in the Large White and Landrace populations, and this, whether affecting the association analysis results or not, should be validated in the future study using a larger size of samples.

In conclusion, we first isolated the porcine *IBP4* gene and performed necessary sequence analysis, polymorphism analysis and association analysis. This established the primary foundation for further insight into this novel pig gene.

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