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RESEARCH ARTICLE

Distribution and linkage disequilibrium analysis of polymorphisms of *GH1* gene in different populations of pigs associated with body size

YUNYUN CHENG[1], SONGCAI LIU[1,2], DAN SU[1], CHAO LU[1], XIN ZHANG[1], QINGYAN WU[1], SIMING LI[1], HAOYU FU[1], HAO YU[1]* and LINLIN HAO[1]*

[1]*College of Animal Science, Jilin University, 5333 Xi'an Road, Changchun, Jilin 130062, People's Republic of China*
[2]*Five-Star Animal Health Pharmaceutical Factory of Jilian Province, 5333 Xi'an Road, Changchun, Jilin 130062, People's Republic of China*

Abstract

Growth hormone (GH) has been considered as a candidate gene for growth and body size in pigs. In this study, polymorphisms of the *GH1 g*ene were evaluated for associations with body size traits in 190 pig individuals. Seventeen single-nucleotide polymorphisms (SNPs) were identified in *GH1* gene of the large pig breeds and miniature pig breeds using direct sequencing and genotyped by allele-specific PCR approach. Notably, six (g.237A>G, g.283T>C, g.309A>G, g.318A>G, g.540A>G and g.544A>G) of them were significantly associated with body size, of which three loci (g.283T>C, g.309A>G, g.318A>G) located in the signal-peptide coding region of *GH1* gene compose a CGG haplotype for large pigs and TAA haplotype for miniature pigs ($P < 0.001$), two loci (g.540A>G and g.544A>G) located in the second intron of *GH1* gene compose a GG haplotype for large pigs and AA haplotype for miniature pigs ($P < 0.001$). Our results demonstrate that these SNPs in *GH1* gene are associated with the body size of pigs providing genetic basis for pig breeding with the improved economic benefits.

[Cheng Y., Liu S., Su D., Lu C., Zhang X., Wu Q., Li S., Fu H., Yu H. and Hao L. 2016 Distribution and linkage disequilibrium analysis of polymorphisms of *GH1* gene in different populations of pigs associated with body size. *J. Genet.* **95**, 79–87]

Introduction

The body size of pig is the most intuitive trait index in pig production and very important in measuring the economic traits in pigs which is mainly decided by body weight, body length, body height and chest circumference. Thus, understanding the effect mechanisms of the pig body size is a key matter in the area of pig breeding and to improve the production performance. The main determinants of body size are growth hormone (GH) and insulin-like growth factors (IGFs) (Favier *et al.* 2001), and GH has pervasive effects on growth rate, behaviour, feeding, metabolism, osmoregulation, smoltification and immunity in fish (Björnsson 1997). GH, a 191-amino acid polypeptide, which is primarily synthesized and secreted in the anterior pituitary has proproliferative, antiapoptotic and metabolic effects in various target tissues (Abdel-Meguid *et al.* 1987). The porcine GH (pGH) gene has been assigned to chromosome 12 band pl.2–1.5. It appears to be a single copy gene. The coding region consists of five exons and four introns, covering a total transcribed area of about 1.7 kb (Vize *et al.* 1988; Yerle *et al.* 1993; Cheng *et al.* 2000). The leader peptide coding region of pGH was 78 bp, and the mature region was 570 bp. Studies have demonstrated that differences in the final body size between medium-sized and giant dog breeds were associated with differences in GH release at young age (Favier *et al.* 2001). Growth rate was substantially higher in GH-transgenic salmonid fish than in wild-type (WT) fish (Leggatt *et al.* 2012). Fast growth in transgenics was associated with markedly higher appetite and feeding motivation than WT fish (Sundstrom *et al.* 2003). In addition, *GH* gene is one of the candidate genes for detecting polymorphisms associated with the growth and carcass traits in chicken, cattle and other animals (Stephen *et al.* 2001; Thakur *et al.* 2006; Shahnaz *et al.* 2008; Ishida *et al.* 2010; Liu *et al.* 2011). Wu *et al.* (2012) showed one novel polymorphism: a variation in intron 2 of *GH* gene (C172T) was associated with some growth and carcass traits in three duck populations, and the TT and CT genotypes were associated with superior growth and carcass traits in carcass weight, dressing percentage and percentage of eviscerated weight. Experts found four novel SNPs in *Siniperca chuatsi GH* gene and of them, three were significantly associated with growth performance.

*For correspondence. E-mail: Linlin Hao, haolinlin@jlu.edu.cn; Hao Yu, yu_hao@jlu.edu.cn.

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This may suggest that these SNPs could affect growth performance in *S. chuatsi*, and can be employed in marker-assisted selection (MAS) for the purpose of advancing *S. chuatsi* breeding and genetic research (Tian *et al.* 2014). Many studies have also shown a close relationship between *bGH* gene polymorphisms and productive traits in dairy cattle (Lucy *et al.* 1993; Lagziel *et al.* 1996; Yao *et al.* 1996; Grochowska *et al.* 2001). Cheong *et al.* (2006) showed that SNPs in GH affect growth rate, carcass weight, longissimus muscle area and fatty acid composition. Li *et al.* (2006) showed that GH peptide sequence of mature protein in Rongjiang pig was different from that of four western meat-type breeds and eight local breeds at the residue Ile108 substituted by Val108 and that may be a reason for the small body size of the Rongjiang pigs. Deng *et al.* (2011) demonstrated that the S104P mutation was found in the mature GH protein of Bamaxiang pigs. All above aspects suggest that there is some correlation between SNPs of *GH1* gene and body size. Therefore, this study was aimed to identify and characterize polymorphisms of GH gene in two body size pig breeds and to ascertain whether the SNPs in *GH1* gene have association with the pig body size thereby provide basic theory for the improvement of genetic characters of the pigs.

Materials and methods

Samples and DNA extraction

Samples were taken from individuals that were collected from three large pig breeds (body weight >190 kg, body length >140 cm, body height >75 cm and chest circumference >140 cm for adult) (Chinese livestock or poultry genetic resource-pigs 2011): large white pigs (48), northeast wild pigs (48) and Junmu no.1 pigs (24); two miniature pig breeds (body weight <45 kg, body length <85 cm, body height < 45 cm and chest circumference <85 cm for adult) (Chinese livestock or poultry genetic resource-pigs 2011): Bama xiang pigs (40) and Tibetan mini-pigs (30), totally 190 samples. Genomic DNA was extracted from liver and kidney of the pigs according to the manufacturer's introductions of Multisource Genomic DNA Miniprep kit (AxyGen, Hangzhou, China).

GH gene PCR from the genomic DNA pool of each pig breeds

The genomic DNA of all pig individuals for each pig breeds were mixed with equal amount as the DNA pool. Based on the sequence of *GH1* (GenBank NC_010454.3), two pairs of primers (table 1) were designed by Primer Primier 5 (Premier Biosoft International, Palo Alto, USA) to produce the overlapping fragments, totally 1934 bp, covering the complete *GH1* gene. The PCR were carried out in a reaction volume of 20 μL containing 50 ng of the genomic DNA pool of the each pig breeds (Bama xiang pigs, Tibetan mini-pigs, Junmu no.1 pigs, northeast wild pigs and large white pigs), 2 μL 10$\times$ PCR buffer, 0.5 μL each of 10 μM forward and reverse primers, 2 μL of 10 mM dNTPs and 1 unit *Ex Taq* polymerase (TaKaRa Biotechnology, Dalian, China). PCR was performed using the programme: 8 min denaturation at 95°C; 30 amplification cycles, each of 30 s at 95°C (denaturation), 30 s at the optimized annealing temperature (table 1), 45 s at 72°C (extension); and 8 min final extension at 72°C. The target fragments were separated on 1% agarose gel. The PCR products were sequenced by Genewiz (Beijing, China). Seventeen SNPs were screened by the sequence alignments (Dnastar, Madison, USA) of the five pig breeds.

SNPs genotyping and chi-square test

The SNPs were genotyped by the method of allele-specific-PCR (AS-PCR). This target amplification method is based on positioning the 3′ base of a PCR primer to match one SNP allele and only accurately extend the correctly matched primer under stringent conditions. Therefore, for each SNP locus, two forward primers and a communal reverse primer were designed (table 2). The genotypes of each SNP were assessed by the separation of the two amplified products on a standard 2.0% agarose gel. The genetic diversity parameters such as the genotype frequency and allelic frequency were determined by direct counting according to the genotyping result (table 3), the gene heterozygosity (H_e), gene homozygosity (H_o) were a measure of genic variation of a population and were calculated according to Nei's (1973) methods. The polymorphism information content (PIC) was calculated according to Botstein's (Botstein *et al.* 1980) methods. To obtain association between the SNPs and body size, we counted the genotype frequency of each SNP of the large pig breeds and the miniature pig breeds, and a chi-square test (χ^2) was used to test associations of SNPs located in *GH1* gene of two body size pig breeds, a significance threshold for the association analysis was set to $P \leq 0.05$.

Table 1. Primer pairs used for the *GH1* gene amplification.

Primer	Sequences (5′–3′)	Position in gene*	Product lengths (bp)	Annealing temperature (°C)
GH-1	F: ACAGGATGAGTGGGAGGAGGT	(−206) − (−186)	1491	57
	R: GGAGAAGGACAAAGAGGGAAGA	1264–1285		
GH-2	F: AGTTCCTCAGCAGGGTCTTCA	993–1013	814	62
	R: CGGGTCAACCATCATTCAG	1788–1806		

*The A base of the start codon ATG as the first base.

Table 2. Position of identified SNPs in the *GH1* gene of large and miniature pigs and AS-PCR primers.

N	AS-PCR primer sequences (5′–3′)	SNP designation	Single marker association[a] (*P* value)	GenBank accession number for the SNP	Variant location	Amino acid	
						Site	Amino acid
1	F: CCCAGGACCCAGCTCCC/T R: ATTCACCCCTCTCCACCACC	g.-40T>C	0.7838	rs343965842	Upstream		
2	F: GTGGGGGCCATGCAGAC/T R: GGACAGCCATCCAGGTCTACAC	g.76T>C	0.7838	rs324095071	Intron1		
3	F: GGGGGCCATGCAGACG/A R: CCCCTTCAGTTTACACTCACCTG	g.77A>G	0.9183	rs335979542	Intron1		
4	F: ACCTTGGGCTTTGGGGC/T R: GGACAGCCATCCAGGTCTACAC	g.106T>C	0.874	rs342850053	Intron1		
5	F: CGAATGTGAGCATGGATATCTACC/T R: GGACAGCCATCCAGGTCTACAC	g.133T>C	0.874	rs336668269	Intron1		
6	F: TCCCTGGGGGAGGGGA/G R: CTGGGCGTTCTGGATGGAG	g.176A>G	0.068	rs328422878	Intron1		
7	F: GGGATCCCTCTCTCACGG/A R: GGGCGTTCTGGATGGAG	g.237A>G	<0.001	rs338049391	Intron1		
8	F: CCGCGCTCCTGGCTTTT/C R: GCAGCCTTCTTAGGAAGAGGGA	g.283T>C	<0.001	rs341706671	Exon2	13	F
9	F: CTGCCTGCCCTGGACTCA/G R: AAGGCCGCCCAACCAT	g.309A>G	<0.001	rs81214388	Exon2	22	Q/R
10	F: CCTGGACTCAGGAGGTGGA/G R: GGAGAAGGCCGCCCAA	g.318A>G	<0.001	rs340087546	Exon2	25	G/D
11	F: AACCGAAGATGCTATCAGGTG/A R: CTGTTGGTGAAGACCCTGCT	g.540A>G	<0.001	rs335914525	Intron2		
12	F: CGAAGATGCTATCAGGTGAGTG/A R: CAGCGAGAAGCGCAGCA	g.544A>G	<0.001	rs339700679	Intron2		
13	F: GGACGCCCACCGGCA/G R: TTTGTCGTAGGTTTGCTTGAGGA	g.918A>G	0.5333	rs345927174	Intron3		
14	F: CGGCAGAGGCAGCGC/T R: TGTCGTAGGTTTGCTTGAGGATC	g.928T>C	0.872	No	Intron3		
15	F: GCAGAGGCAGCGCCCA/C R: TCGTAGGTTTGCTTGAGGATCTG	g.931A>C	0.5120	rs345927174	Intron3		
16	F: CGTGGGCTGGGGGAGA/G R: ATTAGGAAAGGACAGCAGGCATT	g.1211A>G	0.5477	rs319099860	Intron4		
17	F: GGAGGGGAGGGTGGAAGAT/C R: TTTGTCGTAGGTTTGCTTGAGGA	g.1329T>C	0.4683	rs321252801	Intron4		

[a]Association between the SNPs and body size of pigs.

Table 3. Genetic diversity parameters of *GH1* gene among pig breeds.

Breed	Number	SNPs	Genotype frequency			Allelic frequency		H_o	H_e	PIC
			TT	TC	CC	T	C			
BM	40	g.-40T>C	0.20	0.60	0.20	0.50	0.50	0.40	0.60	0.375
TM	30		0.467	0.400	0.133	0.67	0.33	0.60	0.40	0.344
NW	48		0.091	0.636	0.273	0.40	0.60	0.36	0.64	0.365
NW	24		0.25	0.25	0.50	0.38	0.62	0.75	0.25	0.360
LW	48		0.522	0.434	0.044	0.74	0.26	0.57	0.43	0.311
			TT	TC	CC	T	C			
BM	40	g.76T>C	0.20	0.60	0.20	0.50	0.50	0.40	0.60	0.375
TM	30		0.467	0.400	0.133	0.67	0.33	0.60	0.40	0.344
NW	48		0.091	0.636	0.273	0.40	0.60	0.36	0.64	0.365
JM	24		0.25	0.25	0.50	0.38	0.62	0.75	0.25	0.360
LW	48		0.522	0.434	0.044	0.74	0.26	0.57	0.43	0.311
			AA	AG	GG	A	G			
BM	40	g.77A>G	0.35	0.40	0.25	0.55	0.45	0.60	0.40	0.372
TM	30		0.467	0.4	0.133	0.67	0.33	0.60	0.40	0.344
NW	48		0.091	0.636	0.273	0.40	0.60	0.36	0.64	0.365
JM	24		0.25	0.25	0.50	0.38	0.62	0.75	0.25	0.360
LW	48		0.522	0.434	0.044	0.74	0.26	0.57	0.43	0.311
			TT	TC	CC	T	C			
BM	40	g.106T>C	0.20	0.60	0.20	0.50	0.50	0.40	0.60	0.375
TM	30		0.467	0.400	0.133	0.67	0.33	0.60	0.40	0.344
NW	48		0.1818	0.4546	0.3636	0.41	0.59	0.55	0.45	0.367
JM	24		0.25	0.25	0.50	0.38	0.62	0.75	0.25	0.360
LW	48		0.522	0.391	0.087	0.72	0.28	0.61	0.39	0.322
			TT	TC	CC	T	C			
BM	40	g.133T>C	0.20	0.60	0.20	0.50	0.50	0.40	0.60	0.375
TM	30		0.467	0.4	0.133	0.67	0.33	0.60	0.40	0.344
NW	48		0.1818	0.4546	0.3636	0.41	0.59	0.55	0.45	0.367
JM	24		0.25	0.25	0.50	0.38	0.62	0.75	0.25	0.360
LW	48		0.522	0.391	0.087	0.72	0.28	0.61	0.39	0.322
			A A	A G	G G	A	G			
BM	40	g.176A>G	0.25	0.25	0.50	0.38	0.62	0.75	0.25	0.360
TM	30		0.267	0.533	0.20	0.53	0.47	0.47	0.53	0.374
NW	48		0.273	0.091	0.636	0.32	0.68	0.91	0.09	0.340
JM	24		0.333	0.333	0.334	0.50	0.50	0.67	0.33	0.375
LW	48		0.565	0.348	0.087	0.74	0.26	0.65	0.35	0.311
			AA	AG	G G	A	G			
BM	40	g.237A>G	0.25	0.40	0.35	0.45	0.55	0.60	0.40	0.372
TM	30		0.133	0.533	0.334	0.40	0.60	0.47	0.53	0.365
NW	48		0.000	0.273	0.727	0.14	0.86	0.73	0.27	0.212

Table 3. (contd).

Breed	Number	SNPs	Genotype frequency			Allelic frequency		H_o	H_e	PIC
JM	24		0.000	0.50	0.50	0.25	0.75	0.50	0.50	0.305
LW	48		0.00	0.043	0.957	0.02	0.98	0.96	0.04	0.038
			TT	TC	CC	T	C			
BM	40	g.283T>C	0.30	0.60	0.10	0.60	0.40	0.40	0.60	0.364
TM	30		0.333	0.60	0.067	0.63	0.37	0.40	0.60	0.358
NW	48		0.091	0.364	0.545	0.27	0.73	0.64	0.36	0.317
JM	24		0.667	0.25	0.083	0.80	0.20	0.75	0.25	0.269
LW	48		0.0435	0.4348	0.5217	0.26	0.74	0.57	0.43	0.311
			AA	AG	GG	A	G			
BM	40	g.309A>G	0.30	0.60	0.10	0.60	0.40	0.40	0.60	0.365
TM	30		0.533	0.40	0.067	0.73	0.27	0.60	0.40	0.317
NW	48		0.091	0.4545	0.4545	0.32	0.68	0.55	0.45	0.341
JM	24		0.667	0.25	0.083	0.80	0.20	0.75	0.25	0.269
LW	48		0.0435	0.4348	0.5217	0.26	0.74	0.57	0.43	0.311
			AA	AG	GG	A	G			
BM	40	g.318A>G	0.30	0.60	0.10	0.60	0.40	0.40	0.60	0.365
TM	30		0.533	0.40	0.067	0.73	0.27	0.60	0.40	0.317
NW	48		0.091	0.4545	0.4545	0.32	0.68	0.55	0.45	0.341
JM	24		0.667	0.25	0.083	0.80	0.20	0.75	0.25	0.269
LW	48		0.0435	0.4348	0.5217	0.26	0.74	0.57	0.43	0.311
			AA	AG	GG	A	G			
BM	40	g.540A>G	0.25	0.45	0.30	0.48	0.52	0.55	0.45	0.375
TM	30		0.20	0.667	0.133	0.53	0.47	0.33	0.67	0.374
NW	48		0.0455	0.2272	0.7273	0.16	0.84	0.77	0.23	0.233
JM	24		0.083	0.334	0.583	0.25	0.75	0.67	0.33	0.305
LW	48		0.0435	0.8261	0.1304	0.46	0.54	0.17	0.83	0.373
			AA	AG	GG	A	G			
BM	40	g.544A>G	0.25	0.45	0.30	0.48	0.52	0.55	0.45	0.375
TM	30		0.20	0.667	0.133	0.53	0.47	0.33	0.67	0.374
NW	48		0.0455	0.2272	0.7273	0.16	0.84	0.77	0.23	0.233
JM	24		0.083	0.334	0.583	0.25	0.75	0.67	0.33	0.305
LW	48		0.0435	0.8261	0.1304	0.46	0.54	0.17	0.83	0.373
			AA	AG	GG	A	G			
BM	40	g.918A>G	0.20	0.60	0.20	0.50	0.50	0.40	0.60	0.375
TM	30		0.467	0.133	0.40	0.53	0.47	0.87	0.13	0.374
NW	48		0.2273	0.5455	0.2272	0.50	0.50	0.45	0.55	0.375
JM	24		0.25	0.167	0.583	0.33	0.67	0.83	0.17	0.344
LW	48		0.5217	0.4348	0.0435	0.74	0.26	0.57	0.43	0.311

Table 3. (contd).

Breed	Number	SNPs	Genotype frequency			Allelic frequency		H_o	H_e	PIC
			TT	TC	CC	T	C			
BM	40	g.928T>C	0.20	0.60	0.20	0.50	0.50	0.40	0.60	0.375
TM	30		0.467	0.133	0.40	0.53	0.47	0.87	0.13	0.374
NW	48		0.1818	0.4545	0.3637	0.41	0.59	0.55	0.45	0.366
JM	24		0.25	0.25	0.50	0.38	0.62	0.75	0.25	0.360
LW	48		0.5217	0.3913	0.0870	0.72	0.28	0.61	0.39	0.322
			AA	AC	CC	A	C			
BM	40	g.931A>C	0.20	0.60	0.20	0.50	0.50	0.40	0.60	0.375
TM	30		0.467	0.133	0.40	0.53	0.47	0.87	0.13	0.374
NW	48		0.2273	0.50	0.2727	0.48	0.52	0.50	0.50	0.375
JM	24		0.25	0.25	0.50	0.38	0.62	0.75	0.25	0.360
LW	48		0.5652	0.3478	0.0870	0.72	0.28	0.65	0.35	0.322
			AA	AG	GG	A	G			
BM	40	g.1211A>G	0.80	0.05	0.15	0.82	0.18	0.95	0.05	0.252
TM	30		0.467	0.333	0.20	0.63	0.37	0.67	0.33	0.358
NW	48		0.542	0.25	0.208	0.67	0.33	0.75	0.25	0.344
JM	24		0.333	0.50	0.167	0.58	0.42	0.50	0.50	0.369
LW	48		0.583	0.375	0.042	0.77	0.23	0.62	0.38	0.291
			TT	TC	CC	T	C			
BM	40	g.1329T>C	0.80	0.05	0.15	0.82	0.18	0.95	0.05	0.252
TM	30		0.467	0.333	0.20	0.63	0.37	0.67	0.33	0.358
NW	48		0.542	0.167	0.291	0.63	0.37	0.83	0.17	0.358
JM	24		0.333	0.50	0.167	0.58	0.42	0.50	0.50	0.369
LW	48		0.542	0.417	0.041	0.75	0.25	0.58	0.42	0.305

BM, Bama Xiang pigs; TM, Tibetan mini-pigs; NW, northeast wild pigs; JM, Junmu no.1 pigs; LW, large white pigs;H_o, gene homozygosity; H_e, gene heterozygosity; PIC, polymorphism information content.

MultiSNP haplotype analysis

For the SNPs that associate with the body size according the chi-square, the haplotype test was conducted with the genotypes assessment to identify if a haplotype was more informative than a single SNP. Among them, the ones that follow Hardy–Weinberg equilibrium (HWE) $P > 0.01$ were included in the haplotype analysis. The haplotype test and linkage disequilibrium (LD) structure as measured by D′ and r^2 was performed with the HaploView software (Daly Lab at the Broad Institute Cambridge, USA, ver. 3.32) (Barrett *et al.* 2005).

Results

SNPs loci in five pig breeds

Seventeen SNPs were found by comparing the genome sequence of the five pig breeds (table 2). Sixteen were previously annotated in GenBank and g.928T>C is a novel SNP located in intron 3. At least two genotypes were detected at all SNPs loci. Three SNPs (g.283T>C, g.309A>G, g.318A>G) were detected in the signal-peptide-coding region of *GH* gene and all others were in the introns (figure 1). In addition, the 309A (large pigs) > G (miniature pigs) led to the residue Gln replaced by Arg and the SNP at position 318 A (large pigs) > G (miniature pigs) led to the residue Gly replaced by Asp.

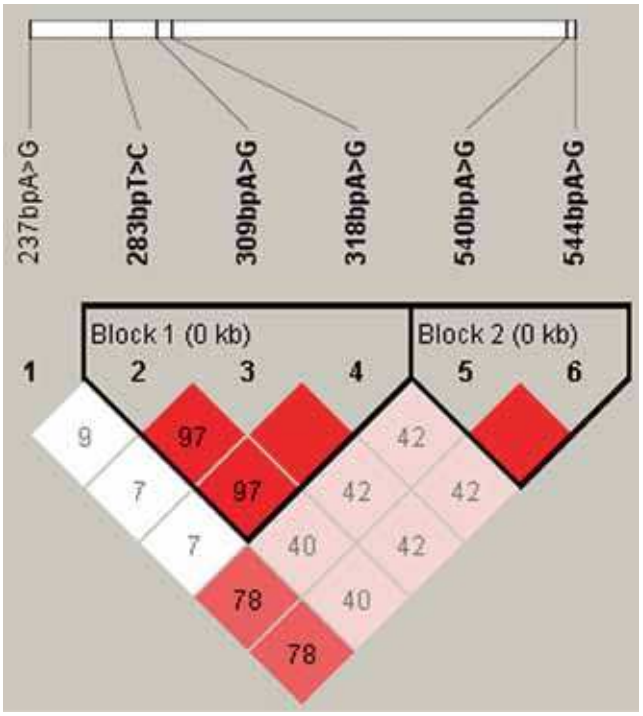

Figure 2. SNPs and LD pattern of pig *GH1* gene in the large and miniature pig breeds. LD blocks are marked with triangles. Values in boxes are LD (r^2) between SNP pairs and the boxes are coloured according to the standard Haploview colour scheme: LD>2 and D′ = 1, red; LD > 2 and D′ < 1, shades of pink/red; LD < 2 and D′ = 1, white.

Diversity analysis

Genetic indices (H_o, H_e and PIC) in these five pig breeds are presented in table 3. The values of H_o (gene homozygosity) and H_e (gene heterozygosity) were nearing to 0.5. The minimum and maximum PIC values were 0.038 and 0.375, respectively. According to the classification of PIC value (PIC value < 0.25, low polymorphism; 0.25 < PIC value < 0.5, intermediate polymorphism; and PIC value > 0.5, high polymorphism), these pig populations mainly belonged to intermediate polymorphism in these SNPs. This reflected that there was an intermediate genetic diversity within pig *GH1*

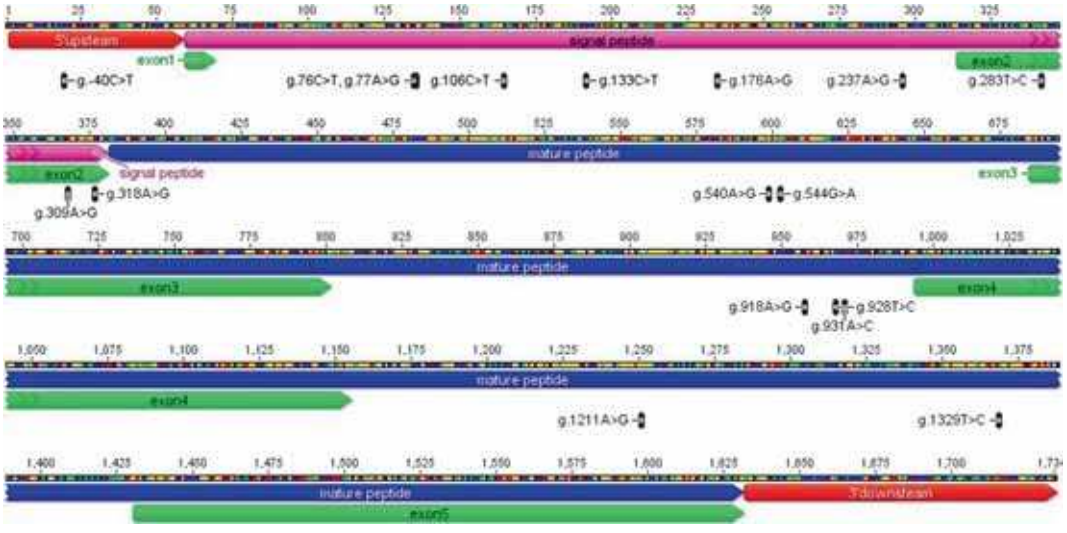

Figure 1. SNPs located in *GH1* gene among the large and miniature pig breeds.

Table 4. Haplotypes frequency of block 1 and associations with body size.

Haplotypes	Haplotype frequency		*P*
	Large pig	Miniature pig	
TAA	0.3286	0.7106	<0.001
CGG	0.6571	0.2719	<0.001
CAA	0.0000	0.0175	0.2666
Others	0.0143	0.0000	

Table 5. Haplotypes frequency of block 2 and associations with body size.

Haplotype	Haplotype frequency		*P*
	Large pig	Miniature pig	
GG	0.5000	0.1579	<0.001
AA	0.5000	0.8421	<0.001

gene in the analysed populations. Genetic diversity is essential for the preservation of adaptive potential of species and improvement of production, potentially high selected breeds.

SNPs genotyping and association test of SNPs with body size traits

The genotype of each individual of the two pig populations was identified according to the agarose gel electrophoresis of AS-PCR products. The genotype frequency of each SNP of large pigs and miniature pigs was counted directly. Of the 17 SNPs identified in the two pig populations, six (g.237A>G, g.283T>C, g.309A>G, g.318A>G, g.540A>G and g.544A>G) were significantly associated with body size phenotype using a chi-square test ($P < 0.001$) (table 2).

Haplotype and LD analysis

Analysing together the markers g.237A>G, g.283T>C, g.309A>G, g.318A>G, g.540A>G and g.544A>G through LD blot analysis, two blocks were detected (figure 2). The first block consisted of g.283T>C, g.309A>G and g.318A>G, was located in the second exon which is a part of the signal peptide of the gene and the haplotype composed of alleles (TAA) was found in higher frequency (71.06%) in the miniature pigs than 32.86% in the large pigs ($P < 0.001$), the haplotype CGG was found in higher frequency (65.71%) in the large pigs than 27.19% in the miniature pigs ($P < 0.001$) (table 4). The second block made up of g.540A>G and g.544A>G was located in the second intron, the haplotype composed of alleles (AA) was found in higher frequency (84.21%) in the miniature pigs than 50.00% in the large pigs ($P < 0.001$), the haplotype (GG) was found in higher frequency (50.00%) in the large pigs than 15.79% in the miniature pigs ($P < 0.001$) (table 5).

Discussion

There are many studies that show *GH* gene was associated with growth, reproduction and metabolism (Breier 1999; Bauman 1999; Scaramuzzi *et al.* 1999). In this study, the population design provided an opportunity to detect the interaction between the *GH* gene and the genetic background of two pig populations for body size traits. The phenotypic differences among the two populations probably reflect their different genetic backgrounds, this may provide basis for molecular markers in genetic studies and pig breeding.

In this study, we first detected the polymorphism of the whole gene sequence of *GH* gene in different body size pigs. Seventeen SNPs (include a noval SNP) were detected in the *GH* gene from 190 pig individuals. All of the 17 SNPs presented median polymorphisms, indicating that these SNPs had large genetic variations and selection potentials. The results of the chi-square test showed that six SNPs: g.237A>G, g.283T>C, g.309A>G, g.318A>G, g.540A>G and g.544A>G were associated with the body size of pigs, implying that the selection pressure on these six loci in the populations was powerful and effective.

A haplotype is a physical arrangement of SNP alleles along chromosome (Olivier 2003) with the availability of high density SNP markers. Haplotypes play an important role in association studies and also provide insight on factors influencing the dependency among SNPs (Liu *et al.* 2008). In this study, based on the six SNP loci that associated with the body size, haplotype test showed CGG haplotype is the advantageous haplotype of the large pigs signal peptide, while that of miniature pigs is TAA. Of the three SNPs in the signal peptide, the g.309A>G lead to the 22nd residue Arg in large pigs replaced by Gln in miniature pigs and the SNP at position g.318A>G lead to 25th residue Asp in large pigs replaced by Gly in miniature pigs, which were located in the splice site of the signal peptide. The signal peptide of the secretory proteins plays an important role in protein synthesis and transportation (Nicchitta and Zheng 1998), suggesting that the haplotype difference may cause the variant synthesis and transportation of GH, thereafter change the body size of pigs. On the other hand, the dominant haplotype of the large pigs in the intron is GG and that for the miniature pigs is AA, but the influence mechanism of *GH1* introns to the pig body size is not clear and need further research.

The results from this study support the concept that *GH* gene is an important modulator associated with the body size in swine and highlight the importance of linking genetic variations that may associate the molecular function of GH with body size in pigs. This research may serve as the first step towards the implementation of pig breeding with the improved economic benefits.

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