Brazilian Journal of Medical and Biological Research (2014) 47(9): 753-758, http://dx.doi.org/10.1590/1414-431X20143945
ISSN 1414-431X

Single-nucleotide polymorphism analysis of *GH*, *GHR*, and *IGF-1* genes in minipigs

Y.G. Tian[1]*, M. Yue[1]*, Y. Gu[1], W.W. Gu[1,2] and Y.J. Wang[1]

[1]Laboratory Animal Center, Southern Medical University, Guangzhou, Guangdong, China
[2]Songshan Lake Pearl Laboratory of Animal Science and Technology Co., Ltd., Guangdong, China

Abstract

Tibetan (TB) and Bama (BM) miniature pigs are two popular pig breeds that are used as experimental animals in China due to their small body size. Here, we analyzed single-nucleotide polymorphisms (SNPs) in gene fragments that are closely related to growth traits [growth hormone (*GH)*, growth hormone receptor (*GHR*), and insulin-like growth factor (*IGF*)-*1*)] in these pig breeds and a large white (LW) control pig breed. On the basis of the analysis of 100 BMs, 108 TBs, and 50 LWs, the polymorphic distribution levels of *GH*, *GHR*, and *IGF-1* were significantly different among these three pig breeds. According to correlation analyses between SNPs and five growth traits – body weight (BW), body length (BL), withers height (WH), chest circumference (CC), and abdomen circumference (AC) – three SNP loci in BMs and four SNP loci in TBs significantly affected growth traits. Three SNP sites in BMs and four SNP sites in TBs significantly affected growth traits. SNPs located in the *GH* gene fragment significantly affected BL and CC at locus 12 and BL at locus 45 in BMs, and also BW, WH, CC, and AC at locus 45 and WH and CC at locus 93 in TBs. One SNP at locus 85 in the BM *GHR* gene fragment significantly affected all growth traits. All indices were significantly reduced with a mixture of alleles at locus 85. These results provide more information regarding the genetic background of these minipig species and indicate useful selection markers for pig breeding programs.

Key words: minipigs; SNP; *GH*; *GHR*; *IGF-1*

Introduction

Dwarfism is caused by a genetic disease that results in a disproportionately short stature. Although the mechanisms of human Laro-type dwarfism, sex-linked dwarfism in chickens, and other dwarfism conditions have been well studied (1), the underlying cause of dwarfism in miniature pigs remains elusive. Growth hormone (*GH*), growth hormone receptor (*GHR*), and insulin-like growth factor-1 (*IGF-1*) are candidate genes for evaluating pig growth traits (2). GH is a polypeptide produced by the adenohypophysis, and is a main factor that promotes somatic growth in vertebrates (3). GH is also involved in morphology, physiology, metabolism, immunology, reproduction, and behavior (4), and it promotes skeletal system growth and facilitates amino acid incorporation during protein synthesis (5). GHR is a member of the type I cytokine receptor family and is a transmembrane receptor for growth hormone, which is related to drip loss (6), body weight (BW) (7,8), and marbling score (9). GHR is expressed in many tissues and mediates the effect of GH. IGF-1 has complex biological functions and plays important roles in cell differentiation and proliferation, animal growth, and metabolism. IGF-1 function is particularly important for embryonic development (10). The porcine *IGF-1* gene is also important for regulating body growth, development, and metabolism. Due to the wide and diverse geographic features of China, many minipig breeds are naturally distributed around the country. Over the last two decades, some of these minipig breeds have been adopted and used as laboratory animals, including Tibetan minipigs (TBs), Guizhou xiang minipigs, Guangxi Bama minipigs (BMs), Wuzhishan minipigs, and Banna minipigs (11). The Laboratory Animal Center of Southern Medical University (China) first imported TBs from Tibet to Guangzhou for laboratory animal research in 2004. The acclimatization and experimental animalization of these minipigs have been completed (12). TB is a unique breed that lives in high altitude environments (13). These pigs, which grow slowly and have thin skin, high meat cutability, and extra-fine muscle fibers, exhibit strong adaptability and resistance to harsh environments (14).

Correspondence: Y.J. Wang and /or W.W. Gu, Laboratory Animal Center, Southern Medical University, 1838 Guangzhou North Road, Guangzhou 510515, Guangdong Province, China. E-mail: yujuewangcn@163.com and/or weiwanggu@yeah.net

*These authors contributed equally to this study.

Received February 25, 2014. Accepted May 12, 2014. First published online August 1, 2014.

Minipig breeding has been partially hindered by the paucity of studies regarding functional genes associated with growth and development (15). BMs and TBs are valuable, rare Chinese species that play a unique role in studying new pig breeds. We previously performed single-nucleotide polymorphism (SNP) analysis and molecular genetics research on *MSTN*, *ESR*, *H-FABP*, *SLA*, *DQA*, *DQB*, and *PPARr-2* genes. In the present study, we analyzed *GH*, *GHR*, and *IGF-1* SNPs by performing DNA sequencing in BMs and TBs to further explore the effects of these genes on growth traits. Large white pigs (LWs) from the United Kingdom, also known as Yorkshire pigs, were used as the control.

Material and Methods

Animals

Animal experiments were performed according to the Guidelines on Animal Care and Use established by the Southern Medical University Animal Care and Use Committee. A total of 100 blood samples from 3- to 8-month-old BMs and 108 blood samples from 3- to 8-month-old TBs were collected at the Laboratory Animal Center, Southern Medical University, Guangzhou. Fifty ear samples from LWs were randomly collected at the Dalingshan Food Company, Dongguan, China. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (USA). The animal use protocol was approved by the Institutional Animal Care and Use Committee of Southern Medical University.

Genomic DNA was extracted using Tiangen genomic DNA extraction kits (Tiangen Biotech, China). The purity and concentration of the DNA samples were determined using agarose gel electrophoresis and ultraviolet spectrophotometry. The samples were diluted to 100 ng/μL and stored at –20°C.

GH, *GHR*, and *IGF-1* gene fragment amplification

Primers for *GH* (Gene ID: 347618782), *GHR* (Gene ID: 347618778), and *IGF-1* (Gene ID: 347618789) gene fragments were designed using the Primer 5.0 software (PREMIER Biosoft, Canada). Polymerase chain reaction (PCR) was carried out in a 50-μL volume containing 50 ng template DNA, 2 μL primers (10 μM each), 25 μL of 2 × MasterMix (0.05 units/μL Taq DNA polymerase, 4 mM $MgCl_2$, 4 mM dNTPs), and double-distilled (dd)H_2O (Aidlab Biotechnologies Co., Ltd., China). PCR reactions were performed under the following conditions: a 3-min hot start at 95°C, 35 cycles of denaturation at 94°C for 30 s, annealing (*GH* at 60°C, *GHR* at 58°C, *IGF-1* at 55°C) for 30 s, extension at 72°C for 90 s, and a final extension at 72°C for 10 min. PCR products were loaded onto a 1% agarose gel and visualized using a gel imaging system (Bio-Rad, USA).

DNA sequencing

The PCR products from pig samples were sequenced by the Invitrogen Trading Company (China).

Data analysis

Polymorphism results were analyzed using DNA STAR Lasergene v7.1, Chromas, Variant Reporter, Popgene32, polymorphism information content (PIC) Calc 0.6, SPSS13.0 (ANOVA, chi-square test), and other biological software by searching SNP loci sites and identifying genotypes.

Results

SNP loci of *GH*, *GHR*, and *IGF-1* gene fragments in three pig breeds

A 221-bp fragment of the *GH* gene, a 254-bp fragment of the *GHR* gene, and a 486-bp fragment of the *IGF-1* gene were amplified by PCR. *GH*, *GHR*, and *IGF-1* gene fragments from the three pig breeds were of the expected sizes.

GH gene fragments revealed five SNP loci (A12G, T45C, G84A, G93A, and C133T) in BMs and TBs, but three SNP loci (A12G, T45C, and G93A) in LWs. Only one SNP locus (A85C) was found in the *GHR* gene fragment of BMs, and no loci were found in the other two pig breeds. In *IGF-1* gene fragments, three SNP loci (A100T, A285G, T300C) were observed in BMs and TBs, whereas two SNP loci (A285G and T300C) were found in LWs. On the basis of the number of SNP loci in these three gene fragments among the three pig breeds, the most diversity was observed in the BM breed, and the least diversity was observed in LWs. The differences were also closely related to their geographical distribution and breeding methods. In general, BMs came from the Guangxi Provinces of China, and they were bred from an individual family with very small numbers and without any selection. TBs have a relatively smaller geographical distribution than BMs. In contrast, LWs are bred on large commercial pig farms with specific selection. The LW samples collected for this experiment came from a limited number of farmers.

GH, *GHR*, and *IGF-1* gene fragments and their genotype frequencies

We statistically analyzed the genotype frequencies of *GH*, *GHR*, and *IGF-1* gene fragments (Tables 1-3, K independent samples test), and the PICs are shown in Tables 1-3. The following PICs were identified at each locus of the *GH* gene fragments from BMs, TBs, and LWs, respectively: 0.372, 0.360, and 0.310 at A12G; 0.373, 0.375, and 0.340 at T45C; 0.122, 0.256, and 0 at G84A; 0.029, 0.211, and 0.374 at G93A; and 0.143, 0.369, and 0 at C133T. The G84A and C133T SNP loci were not found in the LW breed. In the *GH* gene fragment GG was only observed in TBs at locus 84. The dominant allele was G, and no AA was present at locus 93 in BMs. Ninety-four percent GA was found in LWs at locus 93. The

Table 1. Genotype and allele frequency of *GH* gene in three pig breeds.

Breed	Number	Locus	Genotype frequency			Allele frequency		PIC
			AA	AG	GG	A	G	
BM	100	12	0.3500 (35)	0.4100 (41)	0.2400 (24)	0.555	0.445	0.372
TB	108		0.1111 (12)	0.5370 (58)	0.3519 (38)	0.380	0.620	0.360
LW	50		0.7000 (35)	0.0800 (4)	0.2200 (11)	0.740	0.260	0.310
			TT	TC	CC	T	C	
BM	100	45	0.2500 (25)	0.4100 (41)	0.3400 (34)	0.455	0.545	0.373
TB	108		0.2685 (29)	0.4629 (50)	0.2685 (29)	0.500	0.500	0.375
LW	50		0.2800 (14)	0.0800 (4)	0.6400 (32)	0.320	0.680	0.340
			GG	GA	AA	G	A	
BM	100	84	0 (0)	0.1400 (14)	0.8600 (86)	0.070	0.930	0.122
TB	108		0.0556 (6)	0.2592 (28)	0.6852 (74)	0.185	0.815	0.256
LW	50		0 (0)	0 (0)	1.0000 (50)	0	1	0
			GG	GA	AA	G	A	
BM	100	93	0.9700 (97)	0.0300 (3)	0 (0)	0.985	0.015	0.029
TB	108		0.7593 (82)	0.2037 (22)	0.0370 (4)	0.861	0.139	0.211
LW	50		0 (0)	0.9400 (47)	0.0600 (3)	0.470	0.530	0.374
			CC	CT	TT	T	C	
BM	100	133	0.8600 (86)	0.1100 (11)	0.0300 (3)	0.085	0.915	0.143
TB	108		0.2222 (24)	0.4074 (44)	0.3704 (40)	0.426	0.574	0.369
LW	50		1.0000 (50)	0 (0)	0 (0)	0	1	0

BM: Bama miniature pigs; TB: Tibetan miniature pigs; LW large white pigs; PIC: polymorphism information content.

percentages of T at locus 133 were 42.6% in TBs and 0% in LWs. The genotype distribution among the three groups was significantly different ($P<0.05$, chi-square test) for four of the five SNP sites.

For the *GHR* gene, the SNP at A85C was found only in BMs. Both TBs and LWs contain the AA allele in this locus. The genotype distribution among the three groups was significantly different ($P<0.05$, chi-square test).

For the *IGF-1* gene fragment, three SNPs were observed (A100T, A285G, and T300C) in the two minipig breeds, but two SNPs (A285G and T300C) were observed in LWs. AA and TT were present at 98% at locus 285 and locus 300 in LWs, respectively. The percentages of A were 70.5% in BMs and 29.6% in TBs at locus 100, whereas the percentages of A were 24.5% in BMs and 68.1% in TBs at locus 285. The genotype distribution among the three groups was significantly different ($P<0.05$, chi-square test).

Among the three gene fragments, the average observed and expected heterozygosities of the *GH* gene in the TB populations were higher than those of BMs. However, it was lower for the *IGF-1* gene, and no SNP sites were observed in the *GHR* gene of TBs (Table 4).

Relationship between growth traits and *GH*, *GHR*, and *IGF-1* gene polymorphisms

SNPs in the *GH* gene fragment significantly affected body length (BL) and chest circumference at locus 12 and BL at locus 45 in BMs; BW, withers height (WH), CC, and abdomen circumference (AC) at locus 45; and WH and CC at locus 93 in TBs. One SNP was observed at locus 85 in BMs. The *GHR* gene fragment significantly affected all of the growth trait characteristics. All indices were significantly reduced with a mixture of alleles in this locus. These results indicate that this locus has the potential to be used as a breeding selection marker for BMs. SNPs

Table 2. Genotype and allele frequency of *GHR* gene in three pig breeds.

Breed	Number	Locus	Genotype frequency			Allele frequency		PIC
			AA	AC	CC	A	C	
BM	100	85	0.7300 (73)	0.0700 (7)	0.2000 (20)	0.765	0.235	0.295
TB	108		1.0000 (108)	0 (0)	0 (0)	1	0	0
LW	50		1.0000 (50)	0 (0)	0 (0)	1	0	0

BM: Bama miniature pigs; TB: Tibetan miniature pigs; LW: large white pigs; PIC: polymorphism information content.

Table 3. Genotype and allele frequency of *IGF-1* gene in three pig breeds.

Breed	Number	Locus	Genotype frequency			Allele frequency		PIC
			AA	AT	TT	A	T	
BM	100	100	0.4600 (46)	0.4900 (49)	0.0500 (5)	0.705	0.295	0.329
TB	108		0.0463 (5)	0.5000 (54)	0.4537 (49)	0.2963	0.7037	0.330
LW	50		0 (0)	0 (0)	1.0000 (50)	0	1	0
			AA	AG	GG	A	G	
BM	100	285	0 (0)	0.4900 (49)	0.5100 (51)	0.2450	0.7550	0.302
TB	108		0.3796 (41)	0.6019 (65)	0.0185 (2)	0.6806	0.3194	0.340
LW	50		0.9800 (49)	0.0200 (1)	0 (0)	0.990	0.010	0.020
			TT	TC	CC	T	C	
BM	100	300	0.5500 (55)	0.4500 (45)	0 (0)	0.775	0.225	0.288
TB	108		0.4537 (49)	0.5185 (56)	0.0278 (3)	0.713	0.287	0.326
LW	50		0.9800 (49)	0.0200 (1)	0 (0)	0.990	0.010	0.020

BM: Bama miniature pigs; TB: Tibetan miniature pigs; LW: large white pigs; PIC: polymorphism information content.

in the *IGF-1* gene fragment did not affect growth trait characteristics in BMs. However, the SNPs at loci 285 and 300 significantly affected BW, BL, and CC in TBs. These three indices were significantly reduced with the mixture of alleles at these two loci. These two SNPs may be used as breeding selection markers for TBs.

Discussion

The *GH* gene fragment displayed the most diversity among the three gene fragments and pig breeds as demonstrated by the number of SNP loci and PIC values. Only one SNP locus was found in the *GHR* gene fragment of BMs. Although the *IGF-1* gene fragment was selected to evaluate the genetic diversity among pig breeds, our results suggest that *GH* is the best candidate for this purpose.

Polymorphism levels were different among the SNP sites. The average PIC is an ideal index to assess gene fragment polymorphisms. A PIC value >0.5 indicates a highly polymorphic region, $0.25<PIC<0.5$ indicates a moderately polymorphic site, and a PIC value <0.25 signifies a low polymorphic site (16). In the *GH* gene fragment, moderately polymorphic sites were observed at A12G and T45C in BMs; A12G, T45C, G84A, and C133T in TBs, and A12G, T45C, and G93A in LWs. The only moderately polymorphic site found in BMs was at A85C in the *GHR* gene fragment. The three SNP sites in the *IGF-1* gene fragment were moderately polymorphic for BMs and TBs but showed low polymorphism for LWs. However, only low polymorphism was observed in TBs in two previous studies (17-19) using the same *IGF-1* gene fragment. The difference in these studies may be due to the different sample sizes. LW body size is much bigger than that of TBs and BMs (20), and only three moderately polymorphic sites were found in LWs. These results indicate that the values of each SNP site vary among the three pig breeds.

The different SNP sites might differentially affect pig growth traits (21-25). Among the nine SNP sites in these

Table 4. Genetic diversity of *GH*, *GHR*, *IGF-1* genes in 3 pig breeds.

Gene	Population	Number	A	Ae	H_o	H_e
GH	BM	100	2.000	1.4622	0.2138	0.2656
	TB	108	2.000	1.7183	0.3704	0.4038
	LW	50	1.800	1.4818	0.7760	0.2240
GHR	BM	100	2.000	1.7009	0.0645	0.4188
	TB	108	1.000	1.000	0.000	0.000
	LW	50	1.000	1.000	0.000	0.000
IGF-1	BM	100	2.000	1.6575	0.5455	0.4156
	TB	108	2.000	1.6978	0.5156	0.4142
	LW	50	1.6667	1.0135	0.9867	0.0133

A: average number of alleles; Ae: effective allele number; H_o, H_e: observed and expected heterozygosity, respectively; BM: Bama miniature pigs; TB: Tibetan miniature pigs; LW: large white pigs.

three gene fragments, three SNP sites in BMs and four SNP sites in TBs significantly affected growth traits. SNPs in the *GH* gene fragment significantly affected BL and CC at locus 12 and BL at locus 45 in BMs, whereas they affected BW, WH, CC, and AC at locus 45 and WH and CC at locus 93 in TBs. The SNP at locus 85 in the *GHR* gene fragment in BMs significantly affected all of the examined growth traits. All indices were reduced significantly with the mixture of alleles at this locus. These results indicate that this locus has the potential to be used as a breeding selection marker in BMs. SNPs in the *IGF-1* gene fragment did not affect growth characteristics in BMs. However, the SNPs at loci 285 and 300 significantly affected BW, BL, and CC in TBs. These three indices were significantly reduced with the mixture of alleles at these two loci. Therefore, these two SNPs may be used as breeding selection markers for TBs.

Genetic heterozygosity indicates the proportion of the group with site heterozygotes at some loci. The average heterozygosity of loci reflects the level of variation in the genetic structure, and variability is directly correlated with heterozygosity and the ability to adapt to the environment (20). Among the three gene fragments, the average observed and expected heterozygosities of the TB population was higher than those of BMs for the *GH* gene, lower for the *IGF-1* gene, and no SNP sites were observed in the *GHR* gene of TBs.

In summary, the *GH* gene fragment represents the best candidate for SNP analysis among these three gene fragments, and some SNP sites are closely related to growth traits.

Acknowledgments

This research was supported by the Guangdong Natural Science Foundation (#S2013010014720); China Postdoctoral Science Foundation (#2014M550439); the National High Technology Research and Development Program of China (863 Program: #2011AA100304); Science and Technology Planning Project (#2010U1E 00811) of Guangzhou City, Guangdong Province, China; Conditions for scientific research project (#2010B0605 00001, #2011B060300014) of Guangdong Province, China; Key Program for International S&T Cooperation Projects of China (#2011DFA33290); Torch Program (#SQ2012GGHE000003A08), and Development Foundation of Special High-tech Zone (#SQ2011GX07E02680, #SQ2011SF11CO1543).

References

1. Godowski PJ, Leung DW, Meacham LR, Galgani JP, Hellmiss R, Keret R, et al. Characterization of the human growth hormone receptor gene and demonstration of a partial gene deletion in two patients with Laron-type dwarfism. *Proc Natl Acad Sci U S A* 1989; 86: 8083-8087, doi: 10.1073/pnas.86.20.8083.
2. Suzuki K, Nakagawa M, Katoh K, Kadowaki H, Shibata T, Uchida H, et al. Genetic correlation between serum insulin-like growth factor-1 concentration and performance and meat quality traits in Duroc pigs. *J Anim Sci* 2004; 82: 994-999.
3. Figueiredo MA, Fernandes RV, Studzinski AL, Rosa CE, Corcini CD, Junior AS, et al. GH overexpression decreases spermatic parameters and reproductive success in two-years-old transgenic zebrafish males. *Anim Reprod Sci* 2013; 139: 162-167, doi: 10.1016/j.anireprosci.2013.03.012.
4. Devlin RH, Yesaki TY, Biagi CA, Donaldson EM, Swanson P, Chan W. Extraordinary salmon growth. *Nature* 1994; 371: 209-210, doi: 10.1038/371209a0.
5. Bockamp E, Sprengel R, Eshkind L, Lehmann T, Braun JM, Emmrich F, et al. Conditional transgenic mouse models: from the basics to genome-wide sets of knockouts and current studies of tissue regeneration. *Regen Med* 2008; 3: 217-235, doi: 10.2217/17460751.3.2.217.
6. Di Stasio L, Destefanis G, Brugiapaglia A, Albera A, Rolando A. Polymorphism of the GHR gene in cattle and relationships with meat production and quality. *Anim Genet* 2005; 36: 138-140, doi: 10.1111/j.1365-2052.2005.01244.x.
7. Curi RA, Oliveira HN, Silveira AC, Lopes CR. Effects of polymorphic microsatellites in the regulatory region of IGF1 and GHR on growth and carcass traits in beef cattle. *Anim Genet* 2005; 36: 58-62, doi: 10.1111/j.1365-2052.2004.01226.x.
8. Sherman EL, Nkrumah JD, Murdoch BM, Li C, Wang Z, Fu A, et al. Polymorphisms and haplotypes in the bovine neuropeptide Y, growth hormone receptor, ghrelin, insulin-like growth factor 2, and uncoupling proteins 2 and 3 genes and their associations with measures of growth, performance, feed efficiency, and carcass merit in beef cattle. *J Anim Sci* 2008; 86: 1-16, doi: 10.2527/jas.2006-799.
9. Hale CS, Herring WO, Shibuya H, Lucy MC, Lubahn DB, Keisler DH, et al. Decreased growth in angus steers with a short TG-microsatellite allele in the P1 promoter of the growth hormone receptor gene. *J Anim Sci* 2000; 78: 2099-2104.
10. Zhang J, Yang R, Sun S, Sun L, Liu Y, Zhang Y, et al. Cloning and characterization of new transcript variants of insulin-like growth factor-I in Sika deer (*Cervus elaphus*). *Growth Horm IGF Res* 2013; 23: 120-127, doi: 10.1016/j.ghir.2013.04.003.
11. Wang GH, Yin XM, Sun X, Zhao DM. General situation of miniature pig resources at home and abroad. *Chin J Comp Med* 2009; 19: 71-73.
12. Wang YJ, Liu W, Chen C, Yan LM, Song J, Guo KY, et al. Irradiation induced injury reduces energy metabolism in small intestine of Tibet minipigs. *PLoS One* 2013; 8: e58970, doi: 10.1371/journal.pone.0058970.
13. Wang YJ, Wu SJ, Guo KY, Chen C, Xie Q, Gu WW, et al. (1)(8)F-FDG uptake by spleen helps rapidly predict the dose level after total body irradiation in a Tibetan minipig model. *Eur Radiol* 2012; 22: 1844-1851, doi: 10.1007/s00330-012-2451-0.

14. Ryan MT, Hamill RM, O'Halloran AM, Davey GC, McBryan J, Mullen AM, et al. SNP variation in the promoter of the PRKAG3 gene and association with meat quality traits in pig. *BMC Genet* 2012; 13: 66, doi: 10.1186/1471-2156-13-66.
15. Silio L, Rodriguez MC, Fernandez A, Barragan C, Benitez R, Ovilo C, et al. Measuring inbreeding and inbreeding depression on pig growth from pedigree or SNP-derived metrics. *J Anim Breed Genet* 2013; 130: 349-360.
16. Hao LL, Yu H, Zhang Y, Sun SC, Liu SC, Zeng YZ, et al. Single nucleotide polymorphism analysis of exons 3 and 4 of IGF-1 gene in pigs. *Genet Mol Res* 2011; 10: 1689-1695, doi: 10.4238/vol10-3gmr1328.
17. Lang S, Deng JZ, Hao LL, Zeng YZ, Wang J, Li MQ, et al. Single nucleotide polymorphism analysis of IGF-1 gene in some miniature pigs and large breeds. *Chin J Vet Sci* 2011; 31: 750-758.
18. Zambonelli P, Davoli R, Bigi M, Braglia S, De Paolis LF, Buttazzoni L, et al. SNPs detection in DHPS-WDR83 overlapping genes mapping on porcine chromosome 2 in a QTL region for meat pH. *BMC Genet* 2013; 14: 99, doi: 10.1186/1471-2156-14-99.
19. Zhang GW, Gao L, Chen SY, Zhao XB, Tian YF, Wang X, et al. Single nucleotide polymorphisms in the FTO gene and their association with growth and meat quality traits in rabbits. *Gene* 2013; 527: 553-557, doi: 10.1016/j.gene.2013.06.024.
20. Xiao W, Yun P, Li GB, Hang GY, Lu YQ, Wang CD. A comparative study of different sources of growth curve of large white pigs. *J Anhui Agri Sci* 2007; 35: 2912-2913.
21. An X, Bai L, Hou J, Zhao H, Peng J, Song Y, et al. Molecular cloning, tissue expression and SNP analysis in the goat nerve growth factor gene. *Mol Biol Rep* 2013; 40: 857-863, doi: 10.1007/s11033-012-2126-6.
22. Aslam ML, Bastiaansen JW, Elferink MG, Megens HJ, Crooijmans RP, Blomberg lA, et al. Whole genome SNP discovery and analysis of genetic diversity in Turkey (*Meleagris gallopavo*). *BMC Genomics* 2012; 13: 391, doi: 10.1186/1471-2164-13-391.
23. Badke YM, Bates RO, Ernst CW, Schwab C, Fix J, Van Tassell CP, et al. Methods of tagSNP selection and other variables affecting imputation accuracy in swine. *BMC Genet* 2013; 14: 8, doi: 10.1186/1471-2156-14-8.
24. Wang J, Wang H, Jiang J, Kang H, Feng X, Zhang Q, et al. Identification of genome-wide copy number variations among diverse pig breeds using SNP genotyping arrays. *PLoS One* 2013; 8: e68683, doi: 10.1371/journal.pone.0068683.
25. Wang L, Liu X, Zhang L, Yan H, Luo W, Liang J, et al. Genome-wide copy number variations inferred from SNP genotyping arrays using a Large White and Minzhu intercross population. *PLoS One* 2013; 8: e74879, doi: 10.1371/journal.pone.0074879.