Mol Biol Rep (2010) 37:467–471
DOI 10.1007/s11033-009-9644-x

SINE indel polymorphism of *AGL* gene and association with growth and carcass traits in Landrace × Jeju black pig F_2 population

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Received: 31 May 2009 / Accepted: 21 July 2009 / Published online: 1 August 2009

Abstract Genetic polymorphisms in the *glycogen debrancher enzyme* (*AGL*) gene were assessed with regard to their association with growth and carcass traits in the F_2 population crossbred Landrace and Jeju (Korea) Black pig. Three genotypes representing the insertion and/or deletion (indel) polymorphisms of short interspersed nuclear element were detected at frequencies of 0.278 (*L/L*), 0.479 (*L/S*), and 0.243 (*S/S*), respectively. The *AGL S* allele-containing pigs evidenced significantly heavier body weights at birth, the 3rd week, 10th week, and 20th week during developmental stages and higher average daily gains during the late period than were noted in the *L/L* homozygous pigs ($P < 0.05$), respectively. However, average daily gains during the early period were not significantly associated with genotype distribution ($P > 0.05$). With regard to the carcass traits, the *S* allele pigs (*S/-*) evidenced significantly heavier carcass weights and thicker backfat than was measured in *L/L* homozygous pigs ($P < 0.05$). However, body lengths, meat color, and marbling scores were all found not to be statistically significant ($P > 0.05$). Consequently, the faster growth rate during the late period and backfat deposition rather than intramuscular fat deposition cause differences in pig productivity according to genotypes of the *AGL* gene. These findings indicate that the *AGL* genotypes may prove to be useful genetic markers for the improvement of Jeju Black pig-related crossbreeding systems.

Keywords AGL · SINE · Indel · Polymorphism

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Introduction

In the livestock industry, the selection of animals with faster growth rates, better body compositions, and better meat quality is crucial for the production of high-grade meats. Recent advances in molecular biology techniques, such as DNA sequencing and mutation mining, facilitate the detection of genetic polymorphisms and aid in the development of potential markers associated with or linked to desirable characteristics of livestock breeds, including meat quality traits such as intramuscular fat deposition.

The *AGL* gene encodes for the glycogen debrancher enzyme consisting of 35 exons in humans, which evidences 2 catalytic activities: amylo-1,6-glucosidase and 4-alpha-glucanotransferase. Together with phosphorylase, AGL functions as a critical enzyme in carbohydrate metabolism and directs the complete degradation of glycogen [1–3]. Functional deficiency of AGL activities induces an autosomal recessive disease; glycogen storage disease type III

(GSDIII) is characterized by hepatomegaly, hypoglycemia, short stature, growth retardation, variable myopathies, and cardiomyopathy in humans [4]. Mutations associated with GSDIII have been reported in ethnic populations [5–11]. In canines, a single nucleotide deletion in *AGL* exon 32 introduces a frameshift and truncation of the protein and induces GSDIII in curly retrievers [12]. A new alternative splicing isoform mRNA was documented in equine skeletal and heart muscle and novel SNPs [13]. In the pig, *AGL* is located on *Sus scrofa* chromosome (SSC) 4q and two polymorphisms have been described via SINE-indel and *Ava*II-RFLP. *Ava*II genotypes were polymorphic in all eight of the tested pig breeds, but the SINE indel evidenced breed-specific distribution in some breeds, including the Large White and Pietrian [14].

Recently, the potential quantitative trait loci (QTL) for porcine meat quality and productivity were described on SSC4, SSC6, and SSC7 [15–18], but the major QTL remain unclear, because many pig breeds have different genetic backgrounds, and molecular markers have been developed only from a few of the broadly distributed industrial pig breeds such as the Large White, Duroc, Landrace, and Chinese Meishan. Using native Korean pig breeds, previous molecular studies have described the relationships between genetic polymorphisms and economic traits [19–21]. the meat quality-related QTL on SSC4 was described in Landrace × Korean Native pig breeds (KNP) reared in the Korean Peninsula [17]. We reported on the relationships among the genetic polymorphisms of four candidate genes, including *FABP3* encoding on SSC4 and growth traits in the F_2 population generated between Landrace × Jeju Black pigs (JBP) reared only on Jeju Island, Korea [22]. Meat quality and productivity might be associated principally with glycogen metabolism; however, thus far no reports have been published regarding the relationship between the polymorphisms and phenotypic traits in pigs. Therefore, this study assessed the association between the *AGL* genotypes generated by SINE indel and growth and carcass traits in the Landrace × JBP F_2 population.

Materials and methods

Animals and DNA isolation

Blood, muscle or tail samples were collected from F_2 animals generated via reciprocal intercrosses among F_1 crossbreds which produced between 17 (8 boars and 9 sows) Landrace and 19 (8 boars and 11 sows) JBP founders. All experimental procedures were conducted at the Jeju-Substation of the National Institute of Animal Science, Rural Development Administration of Korea. A total of 415 F_2 animals were utilized for the association test, except in certain cases (stillbirth, mummy and early death in before lactation). Growth traits were measured according to developmental stages during production. All carcass traits were determined in accordance with the legal grading standard parameters endorsed by the professional meat quality graders of the Animal Products Grading Service in Korea, as well as the measurement of several additional traits, including backfat and body length, by the authors. Genomic DNA was isolated from blood and muscle via the sucrose–proteinase K method, with slight modification, and utilized as a template for polymerase chain reaction (PCR) amplification.

PCR amplification, genotyping, and DNA sequencing

Enzymatic amplification by PCR utilized primers for the *AGL* gene (aglF: 5′-CTCAACCTTTGGGAGGTATGT-3′ and aglR: 5′-GCTCAGCGAACAGTGACAATA-3′) as previously reported by [14]. PCR was conducted using 20 ul of reaction mixture, including 100 ng of DNA, 0.5 nmole of each primer, and 1 units of LA-*Taq* DNA polymerase (TaKaRa, Japan). PCR consisted of initial heating at 95°C for 3 min, 35 cycles of 45 s for denaturation at 94°C, 45 s for annealing at 60°C and 90 s for extension at 72°C, followed by a 5 min extension at 72°C. The PCR products were separated on agarose gels and visualized via UV illumination, and then genotyped. Genotypes for *AGL* were identified via insertion/deletion patterns (*L*, inserted and *S*, deleted) according to [14]; the L and S alleles evidenced 1,467-bp and 1,192-bp bands on the agarose gels, respectively. Following the purification of the PCR products, for each homozygous genotype the PCR amplified fragments from three individuals were selected and sequenced with an ET Dye-Terminator Sequencing kit (Amersham Pharmacia, USA) with a MegaBace1000 automatic DNA sequencer (Amersham Pharmacia, USA).

Statistical analysis

Allele frequencies for *AGL* indel were calculated using the CERVUS 3.0.3 program [23]. Growth traits included weights (at birth, BWB; 3rd week, BW3; 10th week, BW10; 20th week, BW20), average daily gains (early duration from 3 to 10 weeks, eADG; late duration from 10 to 20 weeks, lADG), and carcass traits included carcass weight (BWC), backfat thickness (BF), loin muscle area (LMA), marbling score (MS), meat color (MC), and body length (BL). The carcass data were collected within 24 h postmortem. Traits and genotypes were statistically analyzed via least squares analysis of variance using the General Linear Model procedure (PROC GLM) of SAS [24]. The Duncan's multiple range test from PROC GLM

was utilized to separate the means. Results were considered significant at $P < 0.05$.

Results and discussion

Allele frequency and genotype distribution

Genetic polymorphisms were detected in F_2 animals crossbred between Landrace and JBP. Table 1 showed the allelic distribution and frequencies of the *AGL* gene in the founder breeds, Landrace and JBP, F_1, and F_2 animals. In the founder breeds, the *AGL* gene was polymorphic in both parents. In the F_2 population, three genotypes of the *AGL* gene were also detected at frequencies of 0.278, 0.479, and 0.243 for *L/L*, *L/S*, and *S/S*, respectively. However, genotype distribution was not significantly related to Hardy-Weinberg equilibrium (data not shown), thereby suggesting artificial planning for intercrossing among F_1 animals.

AGL genotype and growth traits

For the *AGL* gene, the F_2 animals harboring the *S* allele evidenced significantly heavier body weights at the 10th and 20th week than those of the *L/L* homozygotes ($P < 0.05$), respectively, but at birth only the *SS* homozygotes evidenced significantly heavier body weights than those of the *L/-* pigs ($P < 0.05$) (Table 2). For average daily gains, lADG was statistically significant, with the *S* allele group evidencing higher levels than the *L/L* homozygotes ($P < 0.05$), but this was not the case with eADG. This result demonstrated that the pigs harboring the *AGL S* allele evidenced heavier or similar body weights at birth, but they grew more rapidly than the *L/L* homozygote pigs, particularly during the late rearing period after 10 weeks after birth, which suggested that the *AGL S* allele may contribute to increased body weight in the later period of pig production.

Table 1 Genotypes and frequencies of the *AGL* gene in Landrace × Jeju black pig F_2 population

Population	Genotype			Allele	
	L/L	*L/S*	*S/S*	*L*	*S*
JBP (19)	0.158	0.474	0.368	0.395	0.605
Landrace (17)	0.588	0.235	0.177	0.706	0.294
F_1 (69)	0.261	0.638	0.101	0.580	0.420
F_2 (415)	0.278	0.479	0.243	0.517	0.483

Note: Numbers in the parentheses indicate the number of animals tested in this study

Table 2 Mean and SD of traits in Landrace × Jeju black pig F_2 population

Trait[1]	Genotype			Significance
	L/L	*L/S*	*S/S*	
BWB	$1.42^{b} \pm 0.02$	$1.46^{b} \pm 0.02$	$1.56^{a} \pm 0.03$	*
BW3	$5.78^{b} \pm 0.11$	$6.04^{ab} \pm 0.08$	$6.26^{a} \pm 0.12$	*
BW10	$26.50^{b} \pm 0.47$	$27.69^{a} \pm 0.35$	$27.20^{a} \pm 0.5$	*
BW20	$68.76^{b} \pm 0.92$	$73.46^{a} \pm 0.69$	$73.77^{a} \pm 0.97$	ns
eADG	0.42 ± 0.01	0.44 ± 0.01	0.43 ± 0.01	*
lADG	$0.60^{b} \pm 0.01$	$0.65^{a} \pm 0.01$	$0.67^{a} \pm 0.01$	*
BWC	$82.31^{b} \pm 1.15$	$86.28^{a} \pm 0.86$	$87.02^{a} \pm 1.21$	*
LMA	22.09 ± 0.43	22.1 ± 0.33	22.98 ± 0.46	ns
BF	$34.69^{b} \pm 0.76$	$36.89^{a} \pm 0.57$	$36.62^{a} \pm 0.81$	*
BL	104.45 ± 0.53	105.44 ± 0.4	105 ± 0.56	ns
MC	3.90 ± 0.07	3.98 ± 0.05	3.98 ± 0.07	ns
MS	1.92 ± 0.13	1.96 ± 0.1	1.91 ± 0.14	ns

Note: LS Mean ± SD values in the same row with different letters are significantly different at 5% significance thresholds

[1] All abbreviations for each trait are given in the section Materials and methods

AGL genotype and carcass traits

According to the results of association analysis between *AGL* genotypes and carcass traits, the pigs harboring the *S* allele evidenced heavier carcass weights and thicker backfat than the *L/L* homozygote pigs ($P < 0.05$). However, no significant association was detected with the loin muscle area, meat color, body length, and marbling score ($P > 0.05$). Among these factors, the marbling scores represented the levels of intramuscular fat deposition in *M. longissimus dorsi;* consequently, the lack of a detected relation between the *AGL* genotypes and marbling scores indicates that the significant difference in carcass weights according to *AGL* genotypes was not caused by intramuscular fat deposition. On the other hand, because the backfat thickness was significantly associated with *AGL* genotypes, the observed differences in body weights at different developmental stages and at carcass probably caused backfat deposition, but not intramuscular fat deposition.

Conclusion

Thus far, a number of molecular markers have been tested and developed for meat production in pigs. In Korea, the Jeju Black pig has been regarded as a Korean native animal, and Korean people prefer black pork meat, particularly from native black pigs-thus, a genetic improvement program for native black pigs is highly recommended. However, most black pigs in Korea actually appear to be mixed pigs of untraceable origin, because they have been

maintained in farms exposed to exotic pig breeds, including other black-colored pig breeds such as Hampshire and Berkshire pigs. Since the twentieth Century, many industrial exotic pig breeds have been either directly or indirectly imported from other countries in efforts to improve pig production. They have been confused with native pig resources, and most native black pigs in Korea have long since disappeared. At present, small populations of native pigs were separately maintained on the Korean Peninsula (KNP) and Jeju Island (JBP).

In our previous report, genetic polymorphisms of *FABP3* were associated with early stage body weights, and those of *MC4R*, *FABP3*, *MYL2* and *ADCYAP1R1* were associated with late-stage body weights and body lengths in the Landrace × JBP F_2 population [22]. Among these, the *FABP3* gene was also encoded on SSC4, which harbors the *AGL* gene tested in this study. The results of association analysis between *AGL* genotypes and growth traits were shown to be similar to those of *FABP3* analysis; a significant association with body weights and average daily gain was noted during the late period, but not with body length. The potential QTL for meat quality was the first to propose on SSC4 in the pig [15]. However, molecular reports have suggested that the SSC6 be the major QTL containing chromosome that determines the levels of intramuscular fat deposition from the studies of Landrace × KNP intercross [17, 18] rather than SSC4.

According to the results of statistical association analyses of *AGL* genotypes with growth and carcass traits in the Landrace × JBP F_2 population, the pigs harboring the *AGL S* allele evidenced heavier weights, faster growth in the late production period, and thicker backfat than those of the *L/L* homozygous pigs. These findings indicate that the *S* allele AGL protein may be more efficient for glycogen-related metabolism for pig productivity than those of the *L* allele. However, no comparative functional analyses of *AGL* proteins containing each allele have yet been conducted, and thus further studies of the molecular function of this protein in glycogen metabolism and its related development in the pig should definitely be conducted. The present findings of significant associations of *AGL* genotypes with phenotypic traits also indicate that it is possible to use this genetic approach to improve the meat productivity or quality of JBP-related populations using this molecular marker for sire-based predictions. More breed combination studies using the JBP breed and genome-scale molecular approaches will be required in order to develop molecular marker-based improvement systems.

Acknowledgments This study was been supported by Post Doctoral Program Course of National Institute of Animal Science, Rural Development Administration, Republic of Korea.

References

1. Gordon RB, Brown DH, Brown BI (1972) Preparation and properties of the glycogen-debranching enzyme from rabbit liver. Biochem Biophys Acta 289:97–107
2. White RC, Nelson TE (1974) Re-evaluation of the subunit structure and molecular weight of rabbit muscle amylo-1, 6-glugosicase-4-alpha-glucanotransferase. Biochem Biophys Acta 365:274–280
3. Taylor C, Cox AJ, Kernohan JC, Cohen O (1975) Debranching enzyme from rabbit skeletal muscle. Purification, properties and physiological role. Eur J Biochem 51:105–115
4. Howell RR, Williams JC (1983) The glycogen storage disease. In: Stanbury JB, Fredrickson DS, Goldstein JL, Brown MS (eds) The metabolic basis of inherited disease, 5th edn. McGraw Hill Book Company, New York, pp 141–166
5. Shen J, Bao Y, Chen Y-T (1997) A nonsense mutation due to a single base insertion in the 3-prime-coding region of *glycogen debranching enzyme* gene associated with a severe phenotype in a patient with glycogen storage disease type IIIa. Hum Mutat 9:37–40
6. Okubo M, Horinishi A, Makamura N, Aoyama Y, Hashimoto M, Endo Y, Murase T (1998) A novel point mutation in an acceptor splice site of intron 32 (IVS32 A-12-to-G) but no exon 3 mutations in the *glycogen debranching enzyme* gene in a homozygous patient with glycogen storage disease type IIIb. Hum Genet 102:1–5
7. Okubo M, Horinishi A, Takeuchi M, Suzuki Y, Sakura N, Hasegawa Y, Igarashi T, Goto K, Tahara H, Uchimoto S, Omichi K, Kanno H, Hayasaka K, Murase T (2000) Heterogeneous mutations in the *glycogen-debranching enzyme* gene are responsible for glycogen storage disease type IIIa in Japan. Hum Genet 106:108–115
8. Hadjigeorgiou GM, Comi GP, Bordoni A, Shen J, Chen Y-T, Salani S, Toscano A, Fortunato F, Lucchiari S, Bresolin N, Rodolico C, Piscaglia MG, Franceschina L, Papadimitriou A, Scarlato G (1999) Novel donor splice site mutations of *AGL* gene in glycogen storage disease type IIIa. J Inherit Metab Dis 22:762–763
9. Shaiu W-L, Kishnani PS, Shen J, Liu H-M, Chen Y-T (2000) Genotype-phenotype correlation in two frequent mutations and mutation update in type III glycogen storage disease. Mol Genet Metab 69:16–23
10. Lucchiari S, Fogh I, Prelle A, Parini R, Bresolin N, Melis D, Fiori L, Scarlato G, Comi GP (2002) Clinical and genetic variability of glycogen storage disease type IIIa: seven novel *AGL* gene mutations in the Mediterranean area. Am J Med Genet 109:183–190
11. Endo Y, Horinishi A, Vorgerd M, Aoyama Y, Ebara T, Murase T, Odawara M, Podskarbi T, Shin YS, Okubo M (2006) Molecular analysis of the *AGL* gene: heterogeneity of mutations in patients with glycogen storage disease type III from Germany, Canada, Afghanistan, Iran, and Turkey. J Hum Genet 51:958–963
12. Gregory BL, Shelton GD, Bali DS, Chen YT, Fyfe JC (2007) Glycogen storage disease type IIIa in curly-coated retrievers. J Vet Intern Med 21:40–46
13. Herszberg B, Mata X, Giulotto E, Decaunes P, Piras FM, Chowshary BP, Chaffaux S, Guerin G (2007) Characterization of the equine glycogen debranching enzyme gene (AGL): genomic and cDNA structure, localization, polymorphism and expression. Gene 404:1–9

14. Stratil A, Blazkova P, Kopecny M, Bartenschlager H, Van Poucke M, Peelman LJ, Fontanesi L, Davoli R, Scotti E, Russo V, Geldermann H (2003) Characterization of a SINE indel polymorphism in the porcine *AGL* gene and assignment of the gene to chromosome 4q. Anim Genet 34:146–148
15. Andersson L, Haley CS, Ellergren H, Knott SA, Johansson M, Andersson J, Andersson-Eklund L, Edfor-Lijia I, Fredholm M, Hasson I, Hakansson J, Lundstrom K (1994) Genetic mapping of quantitative loci for growth and fatness in pigs. Science 263:1771–1774
16. Ovilo C, Olivier A, Noguera JL, Clop A, Barrangan C, Varona L, Rodriguez C, Toro M, Sanchez A, Perez-Enciso M, Silio L (2002) Test for positional candidate genes for body composition on pig chromosome 6. Genet Sel Evol 34:465–479
17. Jeon JT, Park EW, Jeon HJ, Kim TH, Lee KT, Cheong IC (2003) A large-insert porcine library with seven fold genome coverage: a tool for positional cloning of candidate genes for major quantitative traits. Mol Cells 16:113–116
18. Lee HY, Choi BH, Kim TH, Park EW, Yoon DH, Lee HK, Jeon KJ, Cehong IC, Hong KC (2003) Linkage map and quantitative trait loci (QTL) on pig chromosome 6. J Anim Sci Technol 45:939–948
19. Kim JH, Park EW, Park JJ, Choi BH, Kim TH, Seo BY, Cheong IC, Lim HT, Oh SJ, Lee JG, Jeon JT (2005) Detection of novel mutations in the *FABP3* promoter region and association analysis with intramuscular fat content in pigs. J Anim Sci Technol 47:1–10
20. Lim HT, Kim JH, Choi BH, Lee SH, Park EW, Kim TH, Cho IC, Oh SJ, Lee JG, Jeon JT (2005) Characterization of phosphatidylinositol glycan, class K (*PIGK*) gene and analysis of association with quantitative traits in pigs. J Anim Sci Technol 47:167–176
21. Li S, Li X, Choi BH, Lee CK, Cho BW, Kim JJ, Kim KS (2009) Linkage disequilibrium and gene expression analyses of *IGF2* gene in Korean native pigs. J Anim Sci Technol 51:9–14
22. Han SH, Shin KY, Lee SS, Ko MS, Jeong DK, Jeon JT, Cho IC (2008) Effects of *ADCYP1R1*, *FABP3*, *FABP4*, *MC4R*, *MYL2* genotypes on growth traits in F_2 population between Landrace and Jeju native black pig. J Anim Sci Technol 50:621–632
23. Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol Ecol 16:1099–1106
24. AS S (1999) SAS/STAT software for PC. Release 8.01. SAS Institute Inc, Cary, NC, USA