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Association between Genotypes of the *Isocitrate Dehydrogenase 3, beta subunit (IDH3B)* Gene and Carcass Traits in an F_2 Crossbred Population of Landrace × Jeju (Korea) Black Pigs

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This study tested the association between genetic polymorphisms of the *isocitrate dehydrogenase 3, beta subunit* (*IDH3B*) gene and economic traits in an F_2 crossbred population of Landrace × Jeju (South Korea) Black pigs. A 304-bp insertion/deletion mutation in promoter region was screened for determining genotypes of the *IDH3B* gene in a total of 1,105 F_2 pigs. Three genotypes (*AA*, *AB*, and *BB*) were identified in the founder, F_1, and F_2 populations. Association analysis showed significant differences in carcass weights (CW), backfat thicknesses in three positions of the body (4^{th}-5^{th} ribs, BF5; 11^{th}-12^{th} ribs, BF12; 13^{th} rib-1^{st} lumbar, BFL), and carcass lengths (CL) ($p<0.05$), but not in meat color (MC), eye muscle area (EMA), or marbling scores (MARB) ($p>0.05$). The F_2 *IDH3B BB* homozygotes showed heavier CW (80.790±0.725 kg) and shorter CL (101.875±0.336 cm) than the other genotypes ($p<0.05$). In addition, the BF levels between the 4^{th} - 5^{th} and 11^{th} - 12^{th} vertebrae were thicker in the carcasses of pigs with the *IDH3B BB* genotype than with the other genotypes ($p<0.05$). These results suggested that genetic variations in the *IDH3B* gene may serve as molecular genetic markers for improving the Landrace × Jeju Black pig crossbreeding systems.

Key words : Association, carcass trait, genotype, *IDH3B*, Jeju Black pig

Introduction

The Jeju Black pig (JBP), one of native pig breed in South Korea, has been raised on the Jeju Island. It has unique genetic properties differing from those of the mainland pig populations because it is raised on this island that has been isolated for more than a thousand years. The JBP has uniformly black coat color, and shows low growth rate and high level of backfat thickness comparing to those of Western commercial pig breeds. In the carcass grading system of South Korea, the level of backfat thickness is regarded as one of the major factor for determining the quantitative grades of the pork meat. However, it has excellent meat quality characteristics such as white colored fat, rich meat juice, red meat color, and good marbling [1, 3, 10]. In spite of its commercial weaknesses in meat productivity, JBP and its related crossbred populations are preferred by breeders choosing the JBP as final sire in crossbreeding production system in pig industry due to their higher meat quality and disease tolerance.

IDH3B encodes the beta subunit of nicotinamide adenine dinucleotide (NAD)-specific isocitrate dehydrogenase (IDH) which catalyzes the oxidative decarboxylation of isocitrate into alpha-ketoglutarate in the Krebs cycle in mitochondrion. The Krebs cycle is a central pathway of oxidative phospholylation metabolism, where fatty acid was catabolized into Acetyl-CoA during ATP production by the respiratory complexes [4-6, 14]. Recently, Ren et al. [11] documented that the *IDH3B* transcript have been found as differential expression in intercross population between Large White and Meishan, and an insertion/deletion mutation in promoter region of *IDH3B* gene might induce the increase of gene expression which highly related with the levels of backfat thickness according to their genotypes. However, the genotype effects of *IDH3B* gene had been not evaluated in JBP and related populations. In order to look for the effect of genetic variations of *IDH3B* gene on the phenotypic differ-

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ences especially in backfat thickness, this study examined the association between the genotypes and carcass traits in the crossbred F_2 population between Landrace and JBP.

Materials and Methods

Animals and genomic DNA preparation

The F_2 population was produced via reciprocal intercrosses between Landrace and the Jeju Black pig [3]. Muscle and blood samples were prepared from the F_2 progeny for the genomic DNA isolation. Genomic DNA was isolated from the blood and tissue samples with a slightly modified sucrose-proteinase K method [13] and used as a template for polymerase chain reaction (PCR). The association test used the 1,105 F_2 animals. All carcass traits as well as other additional traits were measured, including eye muscle area and marbling scores, in accordance with legal grading standard parameters endorsed by professional meat-quality graders of the Animal Products Grading Service of Korea. The study was conducted in accordance (approval number 2015-0023) with recommendations described in "The Guide for the Care and Use of Laboratory Animals'' published by the Institutional Animal Care and Use Committee of the Jeju National University, Republic of Korea.

PCR amplification and genotyping

A reference nucleotide sequences (Sscrofa10.2:17:37445726:37451909:-1) were obtained from the Pig Genome Project in ENSEMBL database (http://asia.ensembl.org) and used for PCR primer design. PCR primers for amplification of promoter region of *IDH3B* gene were given in Table 1. PCR was performed using 25 ul of reaction mixture including 100 ng of DNA, 1.0 nmole of each primer, and 2.5 units of i-*Taq* DNA polymerase (Intron Biotechnology, South Korea). PCR conditions included initial heating at 95˚C for 5 min, 35 cycles of 45 s for denaturation at 94˚C, 30 s for annealing at 60˚C, and 60 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 by UV-illumination. After purification of PCR products, three primary PCR products from each homozygote for *IDH3B A/A* and *B/B* were directly sequenced using a MegaBACE 1000 automated sequencer (Amersham-Pharmacia, USA). Each genotype was determined based on the differences in lengths due to the insertion/deletion patterns of 304-bp fragment in promoter region. Alleles *A* and *B* were defined as 648-bp and 344-bp PCR amplicons on the gels, respectively, according to those previously reported by Ren et al. [11].

Table 1. Primers for amplification *IDH3B* promoter region

Primer name	Nucleotide sequences
pIDH3B_id304F	CTGAAGCCAGACATACACACC
pIDH3B_id304R	TCATCCTCCTGCGTTAGGTACT

The length of PCR products for each allele *A* and *B* shows 648-bp and 344-bp, respectively.

Data analysis

We calculated allele and genotype frequencies of the *IDH3B* gene using the CERVUS 3.0.3 program [8]. Phenotypic traits included carcass weight (CW), carcass body length (CL), backfat thicknesses (4^{th}-5^{th} ribs, BF5; 11^{th}-12^{th} ribs, BF12; 13^{th} rib-1^{st} lumbar, BFL), meat color (MC), eye muscle area (EMA), total meat muscle area (TMA, *M. longissimus dorsi* + *M. iliocostalis*) and marbling scores (MARB). Carcass data were collected within 24 hours postmortem. The association between genotypes and phenotypic traits were evaluated using the least squares method of General Linear Model procedure, SAS version 8.0 [12]. We used the following model for analysis of the *IDH3B* promoter polymorphism effect:

$$Y_{ij} = \mu + Genotype_i + e_{ij}$$

where Y_{ij} signifies observed traits, μ is the population mean, $Genotype_i$ represents the *IDH3B* genotypes (*A/A*, *A/B*, and *B/B*), and e_{ij} is the random error. We used Duncan's multiple range test from the General Linear Model procedure to separate means, and we considered significance at $p<0.05$.

Results and Discussion

Using the DNA sequencing for promoter region of *IDH3B* gene in founder animals, 304-bp insertion/deletion mutation was also found as like Ren *et al*. [11]. There were no additional mutations found in the 304-bp fragment spanning promoter sequences in Landrace and the JBP populations. The polymorphism of the *IDH3B* 304-bp insertion/deletion was genotyped in the founder, F_1 and F_2 populations (Table 2). In the founder population, *IDH3B* 304-bp fragment inserted allele, *B* is remarkably frequent in JBP (0.974) but 304-bp fragment deleted allele *A* is more frequent in Landrace (0.853) showing similar pattern comparing to those previously reported by Ren et al. [11] which reported a higher frequency in *IDH3B* allele *A* in Western pig breeds, but allele *B* in Asian indigenous pig breeds of China. In addition, as

Table 2. Genotype distribution of 304-bp insertion/deletion polymorphisms in the promoter region of *IDH3B* gene in founder, F_1 and F_2 populations

Population	Genotype			No. of animals	Allele		χ^2	Diversity parameter*		
	AA	*AB*	*BB*		*A*	*B*		Ho	He	PIC
JBP	0.000	0.053	0.947	19	0.0263	0.9737	n.d.	0.053	0.053	0.050
Landrace	0.706	0.294	0.000	17	0.853	0.147	n.d.	0.294	0.258	0.219
F_1	0.055	0.802	0.143	91	0.456	0.544	32.191	0.802	0.499	0.373
F_2	0.213	0.508	0.280	1,105	0.467	0.533	0.364	0.508	0.498	0.374

*, Ho, He, and PIC indicate the values of observed heterozygosity, expected heterozygosity, and polymorphic information content, respectively.

like JBP, the Chinese indigenous pig, Meishan possessing higher frequency of allele *B* and thicker levels of backfat at buttocks than those of Large White. The Landrace used in this study, is a Western pig breed popular for lean meat, thinner backfat, fast growth high productivity, showed higher frequency of allele *A* in genotyping results. In this point of view, we concluded that the genotypic distribution of *IDH3B*, at least in part, coincides with those of phenotypic characteristics of backfat thickness both pig breeds.

Association between the genotypes of *IDH3B* promoter and carcass traits

Table 3 shows the results of the association analyses between the insertion/deletion mutation of promoter region of *IDH3B* and the phenotypic traits recorded from the F_2 population. For the *IDH3B* genotypes, we measured carcass traits and several additional traits including CBL, BF12, BFL, and MMA. Among the carcass traits tested in this study, the five traits, (i.e., CW, CL, BF5, BF12, and BFL) were showed statistical associations according to genotypes of *IDH3B* polymorphisms ($p<0.05$). However, all other traits, MC, MARB, EMA, and TMA were not showed the statistical association with the *IDH3B* genotypes ($p>0.05$).

The F_2 animals carrying the *IDH3B B/B* homozygotes showed relatively heavier body weights for carcass weights (80.790±0.725 kg) than those of the other genotypes (77.807 ±0.825 kg and 78.954±0.534 kg) ($p<0.05$). The F_2 animals possessing *IDH3B A/A* and *A/B* genotypes showed longer CL than those of *B/B* genotypes ($p<0.05$). In addition, the F_2 progeny carrying the *IDH3B B/B* showed significantly larger levels of BF5, BF12, and BFL than those of *A/A* and *A/B* ($p<0.05$).

Putative roles of *IDH3B* gene related to carcass in pigs

Genetic variations provide important bases to explain and analyze phenotypic variation. The statistical associations of the *IDH3B* promoter polymorphisms with CW, CL, and BFs have been found in this study, suggested that the *IDH3B* gene may be involved at least in the fat deposition in subcutaneous fat tissues without relation with intramuscular fat deposition in *M. longissimus dorsi*, and may affect sig-

Table 3. Mean and SE of traits in pig F_2 population

Trait[1]	*IDH3B* genotype			*p*-value	Significance[2]
	AA	*AB*	*BB*		
CW	77.807±0.825	78.954±0.534	80.790±0.725	0.020	*
CL	102.727±0.383	103.084±0.248	101.875±0.336	0.015	*
MC	4.028±0.0551	4.0258±0.036	4.0489±0.049	0.927	n.s.
MARB	1.9227±0.088	1.9202±0.057	1.760±0.0786	0.217	n.s.
EMA	21.498±0.273	21.274±0.176	21.502±0.238	0.668	n.s.
TMA	36.546±0.398	36.567±0.257	37.481±0.348	0.080	n.s.
BF5	33.009±0.503	33.382±0.325	35.697±0.440	1.58E-05	***
BF12	26.872±0.497	27.342±0.321	29.663±0.434	7.74E-06	***
BFL	26.123±0.634	25.568±0.410	27.731±0.554	0.007	**

[1], all abbreviations of each trait are given in the Materials and Methods section.
[2], LS Mean±SE values in the same row are significantly different at 5% (*), 1% (**) and 0.1% (***) significance thresholds, respectively. n.s. indicates not significant.

nificantly higher growth rates related to the CW and CBL in an intercross population between Landrace and JBP. The levels of MARB representing the levels of intramuscular fat deposition in *M. longissimus dorsi* did not show the significant association between the *IDH3B* genotypes and phenotypic differences, whereas the levels of BFs showed significant association. These results proposed that the genetic differences of *IDH3B* gene might be involved only in fat deposition and adipocyte differentiation in the subcutaneous tissues but not in *M. longissimus dorsi*. Ren *et al*. [11] have also described the significant association between the *IDH3B* genotypes and the traits of backfat thickness at buttocks. These findings suggested that *IDH3B* gene may play an important role in fat deposition in tissue-specific manner. Especially, the F_2 animals carried *IDH3B B/B* genotype showed similar patterns of higher levels of BFs and CW, which suggested that the difference of CW might be due to the differences in backfat deposition. On the other hand, the levels of CL among *IDH3B* genotypes were not coincide with those of BFs, indicating that the traits CL and BFs were probably independently affected by *IDH3B* genotypes.

IDH3B, a key enzyme in Krebs cycle is required for energy synthesis in mitochondrion in the cells [4, 5, 7]. In human, the loss of this enzyme by the homozygous frame-shift mutation is only reported in the cases of retinitis pigmentosa in the eye [6]. Therefore, MacDonald *et al*. [9] suggested that it may be possible that the lack of the IDH3 enzymatic activities in mitochondrion might be compensated by the cytoplasmic activities of IDH1 for oxidation of isocitrate and NADH reduction. On the other hand, *IDH3B* has been proposed as a candidate gene for backfat thickness in pig [11]. There were no direct molecular evidences have been reported to reveal the molecular biological backgrounds related to cause the differences of those economic traits, however we hypothesized that *IDH3B* would effect on the weights, body lengths and the levels of backfat thickness via regulation of *IDH3B* protein and efficiency of the Krebs cycle.

Our study provides the hypothesis that the *IDH3B* genotypes may be involved in phenotypic variations of adipogenesis or fat deposition of the pork meat, and productivity related to body weights and body lengths, at least in part through *IDH3B* pathways. We found different effects of this genetic marker associated with economically important traits, but the molecular function of *IDH3B* in the backfat deposition has not been clearly defined until now. Further biochemical and molecular biological experiments will provide more explainable information for the molecular mechanisms of genotype-related functional differences between both *IDH3B* genotypes.

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초록 : 제주흑돼지와 랜드레이스 교배 2세대의 도체형질과 *IDH3B* 유전자형의 상관관계

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본 연구에서는 제주흑돼지와 랜드레이스 품종 사이에서 생산된 F_2 교배 집단의 경제형질과 *isocitrate dehydrogenase 3, beta subunit* (*IDH3B*) 유전자의 유전적 다형성의 상관관계를 시험하였다. *IDH3B* 유전자의 프로모터 지역에서 304-bp 삽입/결실 돌연변이를 기준으로 전체 1,105 F_2들에 유전자형 분석에 이용하였다. 기초축군과 F_1, F_2에서 세 가지 유전자형(*AA*, *AB*, *BB*)이 모두 발견되었다. 통계적 상관 분석결과에서 도체중(CW), 세 지점(4-5번 흉추 사이, 11-12번 흉추 사이, 13번 흉추-1번 요추 사이)에서 측정한 등지방두께와 도체장(CL)의 수준은 유전자형에 따른 유의적인 차이를 나타내었지만(p<0.05), 육색(MC), 등심단면적(EMA)과 근내지방도(MARB)은 유의적인 차이를 나타내지 않았다(p>0.05). *IDH3B* 동형접합자 *BB*를 보유한 F_2 돼지들은 도체중이 더 무겁고(80.790±0.725 kg), 도체장은 더 짧은(101.875±0.336 cm) 양상을 보였다(p<0.05). 또한, *IDH3B* 유전자형 *BB*인 개체들은 *IDH3B AA*나 *AB* 유전자형에 비해 4-5번 흉추 사이, 11-12번 흉추 사이에서 측정된 등지방두께도 더 두꺼운 수준을 나타내었다(p<0.05). 이상의 결과들은 *IDH3B*의 유전적 다양성이 제주흑돼지와 랜드레이스와 관련된 교배육종 체계의 산육능력 향상을 위한 유전적 분자 표지인자로 활용될 수 있음을 나타내었다.