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Associations between polymorphism in the 2,4-Dienoyl-CoA Reductase 1 gene (*DECR*1) and growth traits of Shanxi White pig*

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A polymorphism within exon 2 of the 2, 4-dienoyl-CoA reductase gene (*DECR*1) was investigated by PCR-SSCP in 228 Shanxi White pigs. An association between the *DECR*1 polymorphism and growth traits in Shanxi White pigs was determined with an univariate animal model. The polymorphism was found within exon 2 of the *DECR*1, giving rise to genotypes AA, BB or AB. This polymorphism exhibited a significant effect of generation and sex (P<0.05, P<0.01) on growth traits and backfat thickness. At the age of six months BB pigs showed the body weight and chest girth higher than AA and AB animals. However, the polymorphism revealed no significant effects on other growth traits (P>0.05) though a trend of BB>AB>AA was showed.

KEY WORDS: *DECR*1 / gene polymorphism / growth traits / PCR-SSCP / pig**

2, 4-dienoyl-CoA reductase gene (*DECR*1) encodes for mitochondrial 2,4-dienoyl-CoA reductase 1 (*DECR*1), which serves as an auxiliary enzyme participating in the oxidation pathway of polyunsaturated fatty acids by catalyzing the reduction of trans-2-cis-4-dienoyl-CoA to 3-enoyl-CoA [Kunau *et al*. 1978, Helander *et al*. 1997, Aner *et al*. 1999]. In the pig *DECR*1 is located on chromosome 4 q1.2 (genomic interval 71 to 86 cM), which coincides with a QTL associated with phenotypic variation in

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carcass and growth traits, and metabolism of fatty acid [Perez-Enciso *et al*. 2000, Clop *et al*. 2002, Clop *et al*. 2003]. Pig *DECR*1 is therefore thought to be one of the candidate genes for carcass and meat quality and growth traits [Kunau *et al*. 1978, Clop *et al*. 2002, Davoli *et al*. 2002, Amills *et al*. 2005]. Earlier studies have showed that the expression level of *DECR*1 and the activity of the enzyme are associated with the intermuscular fat, and especially linoleic acid content [Walling *et al*. 2000, Perez-Enciso *et al*. 2000]. It was also revealed that the growth rate of pigs is related to polymorphism within the 2,4-dienoyl-CoA reductase gene [Walling *et al*. 2000, Perez-Enciso *et al*. 2000].

Shanxi White pig is a specialized lean-meat dam strain newly established by a systematic breeding programme. The initial basic population was produced through complex crosses made among the Shanxi local breed of Mashen pig, Taihu pig (Er hua lian sub-strain) and imported Landrace boars. The objective of the present study was to identify polymorphism in the *DECR1* coding sequence in Shanxi pig and investigate whether it is associated with its growth traits.

Material and methods

Animals and their phenotypic traits recorded

A total of 228 pigs from generation 4, 5 and 6 of a systematic breeding programme were used (63 males and 165 females). All the pigs were kept under the same feeding and environmental conditions. The following phenotypic traits of pigs were recorded: body weight at birth and at weaning (age 28 days), body weight, body height (at withers), body length, chest girth and backfat thickness at the last rib level (age 6 months).

Genomic DNA extraction

Genomic DNA was isolated from ear tissue by a standard phenol/chloroform extraction method. The isolated genomic DNA was diluted in TE buffer and stored at -20°C until use.

Polymerase Chain Reaction (PCR) primers and conditions

PCR primers were designed based upon the sequence information provided by Davoli *et al*. [2002a]. The primer sequences were as follows: forward 5'- CGTCTAAGTCC TTCCCACC -3', reverse 5'- GCACCTAAGCTGGACAGAT -3'. The PCR mixture contained 2.5 μL 10×PCR buffer, 2 μL dNTP (2.5 mmol/L each), 1 μL forward and reverse primers (10 μmol/L each), 0.2 μL Taq DNA polymerase (5 U/ μL), 1.0 μL genomic DNA (50 ng/ μL), and 18.3 μL ddH_2O. Following were the PCR conditions: one denaturation step at 95°C for 5 min, followed by 31 cycles of 95°C for 30 s, 61°C for 30 s and 72°C for 30 s, and a final extension step at 72°C for 10 min. The PCR product was examined electrophoretically in a 2% agarose gel.

Polymerase Chain Reaction - Single Strand Confirmation Polymorphism (PCR-SSCP) analysis

5 μL PCR product was mixed with 5 μL loading buffer containing 98% Formamide, 0.025% Bromophenol Blue, 0.025% Xylene Cyanol FF, and 10 mmol/L EDTA. The mixture was denatured at 98°C for 10 min, then immediately put on ice and left for 5 min. The samples were eletrophoresed in 14% polyacrylamide gel (PAGE) at 4°C overnight. The gel was then silver-stained, and the image was taken for SSCP analysis.

Statistical

The assumed model for the data of early phenotypic traits such as body weight at birth and weaning was as follows:

$$y = X\beta + Z_{\alpha}\alpha + Z_{m}m + e$$

where:

y – analysed trait vector;

β – fixed effects (three generation effects, two sex effects, three *DECR1* genotype effects);

α – vector of additive genetic effects;

m – maternal effects;

e – the residual vector and X, Zα and Zm are the linkage matrices of β, α and m, respectively.

The assumed model for the data of phenotypic traits at the age of 6 months was as following:

$$y = X\beta + Z\alpha + bt + e$$

where:

y – analysed trait;

β – fixed effects (three generation effects, two sex effects, three *DECR*1 genotype effects);

α – vector of additive genetic effects;

t – age (day) on the day of measurement of body weight;

b – regression index of the age (day) on the day of measurement against body weight;

e – the residual vector, and X and Z are the linkage matrices of β and α, respectively.

Statistical analysis was performed using WOMBAT software, an univariate analysis programme [Meyer 2007]. SAS (*v*6.12) was used to estimate (co)variance components. Subsequently the average information restricted maximum likelihood (AIREML) was used for iterative computation with a convergence threshold of 10^{-9}. The significance of difference of least squares solutions was calculated using a T test.

Results and discussion

A 127 bp gene specific fragment amplified from exon 2 of *DECR*1 was obtained by PCR.-SSCP analysis and yielded three distinct genotypes: AA, BB and AB (Fig. 1). Allele frequencies were 0.5724 and 0.4276 for A and B, respectively.

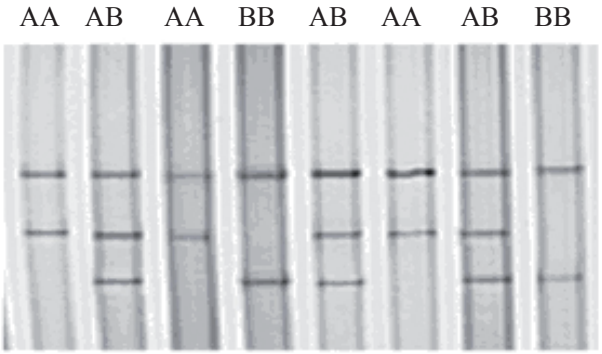

Fig. 1. The representive PCR-SSCP result of the exon 2 of *DECR1*. PCR products were eletrophoresed in 14 % PAGE gel at 4°C overnight, then silver stained.

Table 1. The effects of polymorphism of *DECR*1 gene on body weight and chest girth of Shanxi White pigs at month 6 of age

Genotype	Body weight (kg)			Chest girth (cm)		
	n	mean	GLS±SE*	n	mean	GLS±SE*
AA	62	83.589	0.000 ± 0.000^{B}	62	100.23	0.000 ± 0.000^{B}
AB	126	84.127	0.274 ± 1.266^{B}	125	101.30	0.147 ± 0.834^{B}
BB	29	88.155	3.072 ± 0.870^{A}	27	103.85	2.808 ± 0.247^{A}

*GLS±SE: general least square solutions ± standard errors.
^{AB}Within columns means bearing different superscript letters differ significantly at $P<0.01$.

Statistical analysis revealed that this polymorphism had significant generation effects on body weight and chest girth at month 6 of age (Tab. 1). Animals of genotype BB showed the highest phenotypic parameters of each trait in most of the cases, but highly significant differences ($P<0.01$) between pigs of genotype BB and AB or BB and AA were only observed in body weight and chest girth at the age of 6 months.

All traits related to growth are quantitative polygenetic traits, affected by multiple genetic and environmental factors [Bidanel *et al*. 2002, Kaminski *et al*. 2009]. The basis for the genetic component of quantitative traits is often due to the cumulative effects on the phenotype of many genetic *loci*. Quantitative trait *loci* (QTL) mapping in the pig has identified a number of genomic regions overlapping or close to *DECR*1 *locus* on chromosome 4, which affect growth traits [Magnus *et al*. 2005].

However, causative gene identification and validation is still a daunting task. The 2,4-dienoyl-CoA reductase 1, as a step limiting enzyme in the β-oxidation pathway of polyunsaturated fatty acids, plays a key role in fat and protein metabolism. Therefore, *DECR*1 has been extensively studied as a candidate gene for the control of growth and carcass and meat quality traits in pigs. Studies on the association between the *DECR*1 polymorphism and carcass, meat quality and growth traits [Stefanon *et al*. 2004, Kaminski *et al.* 2009] have produced positive results. The results published here are largely in accordance with the report of Kaminski *et al*. [2009], in which *DECR*1 genotype and growth rate were found to be significantly associated in Polish Landrace boars.

Amills *et al*. [2005] performed an association analysis between two missense SNPs within exon 2 and exon 5 of *DECR*1, and growth, carcass, and meat quality traits in a highly selected Landrace population. Differences in meat quality traits such as isocitrate dehydrogenase activity, *longissimus thoracis* pH, lightness and redness were found among the genotypes. The association between *DECR*1 genotype and other growth traits were not found to be statistically significant [Amills *et al*. 2005]. These findings contrast with our results and others to some extent. The inconsistency between the results could be explained by a difference in population origin and size.

It remains unclear whether the association between polymorphisms of the *DECR*1 gene and growth and meat quality traits are due to changes in enzyme activity and efficiency of beta-oxidation of fatty acids caused by a mutation in the coding sequence of the gene, or that perhaps polymorphisms in *DECR*1 are in linkage disequilibrium with the true causal mutation. However, this does not delay the use of *DECR*1 genotypes as a selection markers in future Shanxi white pig breeding programme.

In summary, our results, together with other studies, indicate a significant association of a *DECR*1 polymorphism with meat quality and growth traits in pigs. This implies that the *DECR*1 polymorphism could be one of the most effective genetic markers applied in marker-assisted selection in Shanxi white pigs.

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